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Vector NTI Suite 7.0 User's Manual

**including
Xpression NTI**

**InforMax, Inc.
7600 Wisconsin Avenue
Bethesda, MD 20814**

Vector NTI Suite7.0 User's Manual, including Xpression NTI

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Chapter 1 Introduction to the Vector NTI Suite 7.0 User's Manual Including Xpression NTI

This Vector NTI Suite 7.0 User's Manual, including Xpression NTI is for users of the Vector NTI/Vector NTI Suite 7.0 and Xpression NTI software packages developed by InforMax, Inc., Bethesda, Maryland. The manual describes the Vector NTI Suite and Xpression NTI software and provides the information on how to optimize their use.

The Vector NTI User

Clients using Vector NTI and/or the Vector NTI Suite and the Xpression NTI components are expected to have some familiarity with window environments and web browsers. Vector NTI uses a convenient graphical user interface (GUI).

It is recommended that Vector NTI and Xpression NTI users at all levels review the three introductory chapters in this user's manual. Their purpose is to familiarize the Vector NTI and/or the Xpression NTI client with the user's manual structure and conventions, the Vector NTI Suite and Xpression NTI software, and the Vector NTI and the Xpression NTI environments.

The Vector NTI Suite User's Manual Format

The manual is written to assist you, the Vector NTI Suite user and or the Xpression NTI user, to master the software quickly, with little reading. To be a useful and simple-to-use resource, it contains:

- Section I: Four introductory chapters, 1-4, covering Vector NTI basics.
- Section II: Vector NTI Suite Tutorial chapters, chapters, 5-17, enabling VNTI users at any level to learn independently how to use components of the Vector NTI Suite.
- Section III: Reference tools, chapters 18 through 26, organized around the functions and features of Vector NTI.
- Section IV: Vector NTI Suite Applications, chapters 27 through 36, covering additional components of the Vector NTI Suite.
- Section V: Xpression NTI, chapters 37 and 38, describing the functions and features of Xpression NTI.
- Section VI: Appendices
- A complete index

Using the manual, you will be able to easily look up and find descriptions of specific features and functions. In addition, the Vector NTI Suite and Xpression NTI Installation Guide will accompany this manual in a separate document. Electronic versions of the Vector NTI Suite 7.0 User's Manual, including Xpression NTI and the Vector NTI Suite, including Xpression NTI Installation Guide are also available at the InforMax Inc. website: <http://www.informaxinc.com/support/tutorials.html>.

Conventions Used in this Manual

The following table uses various typefaces to differentiate between regular text and menu commands, keyboard keys, toolbar buttons, dialog box options and text that you type. The following table describes how conventions are represented in this manual.


<i>Convention</i>	<i>Description</i>	<i>Example</i>
Bold and Capitalized Command Capitalized command > Capitalized command	Menu command Sequential Menu commands	Database > Explore
TEXT IN SMALL CAPS	Keyboard key that you press	The Enter Key ENTER
TEXT IN SMALL CAPS + TEXT IN SMALL CAPS	Keys that you press together	Press SHIFT+ CTRL, then release both.
TEXT IN SMALL CAPS followed by TEXT IN SMALL CAPS	Keys that you press in sequence	Press ENTER, followed by ENTER
Icon	Small picture on a toolbar button that you press	Click the Zoom In button () to enlarge the image
Boldface type	Options or tabs that you select in dialog boxes, list boxes, or drop-down menus Buttons that you click	In the Restriction Enzymes dialog box, select the enzyme(s) and press the Add button.
<i>Italic boldface type</i>	Text that you type	In the New Molecule dialog box, enter the molecule name, <i>my pBR322</i> .
Note:	Highlights a particular concept or extends the current topic	Note: To calculate Separation Time, at least two fragments must be selected.
Important: Or Warning!	Highlights information of which you should be particularly aware	Important: Once parent-descendant relationships have been disconnected, they CANNOT be reconnected.

Table 1. 1 User Manual Conventions

Chapter 2 An Overview of Vector NTI Suite and Xpression NTI

Introduction

Vector NTI Suite a suite of desktop applications, is a comprehensive set of sequence analysis software tools specifically designed for the molecular biologist to study, visualize, manipulate, construct and store biological molecules. With this system, you can:

- Easily navigate and search the user-defined, password-protected molecule and enzyme databases
- Access molecules across a network with user-defined and password-protected databases
- Generate recombinant strategies and protocols and design and analyze PCR primers and other oligos
- Animate virtual gels for restriction fragment analysis
- Analyze physiochemical properties of molecule sequences
- Assemble contigs from text sequences or chromatograms
- Create and customize publication-quality graphics
- Take advantage of full internet connectivity with more than 20 internet sites for searching and importing annotated molecules back into Vector NTI Suite

While all of the Suite components are fully capable of functioning independently of one another, the program integrates data, analyses and presentations easily among the applications.

The desktop expression application, Xpression NTI, is a comprehensive program independent of Vector NTI Suite, designed to store, manage and analyze expression data. With this software, you can:

- Manage expression data in the Expression Database Explorer
- Merge primary gene expression results
- Display graphic representations of expression data
- Filter, sort and cluster expression results for data analysis

Database

The Vector NTI database provides storage and organization functions for molecules and other objects used in cloning strategies, molecule manipulation and analysis, and sequence analysis. Database objects include DNA/RNA and protein molecules, restriction enzymes, oligonucleotides (including PCR primers, sequencing primers and hybridization probes), gel markers. BLAST search results, and citations.

In the Database Explorer window, you can:

- Create new objects
- Edit and delete old objects
- Perform database searches
- Organize objects into convenient groups (subbases)
- Import and export objects
- Create Vector NTI “archives” of objects to share with other users.
- Format references according to bibliographic styles assigned to various professional publications

General database functions let you make backup copies of the database in the specified subfolder of your hard drive or floppy disk, restore all database files from a backup and compress the database by removing all unnecessary data from the datafiles and database tables.

Vector NTI also has two additional data managers:

- Contact Manager to create, modify, and organize the address data of persons or organizations with which you frequently communicate.
- User Field Manager to define data fields to describe your database objects.

Organizing your Biological Objects

Using the Vector NTI database, you can conveniently organize and effectively manage a large amount of molecular biology data. Database objects can be organized into an arbitrary number of *subbases*. The Database Explorer has convenient commands for creating and managing subbases of all database objects.

Description fields of database objects allow you to describe any object property in formal and semiformal ways. Vector NTI serves as a global “organizer” for your biological data.

Database Search

Vector NTI lets you search for database objects using many types of data as search conditions. These include, for example, form (circular/linear), storage type (basic, constructed, translated), size, description/comment/user fields text, and so forth.

Import/Export Functions

You can import molecules (including their feature tables) from GenBank/GenPept, EMBL/SWISS-PROT and FASTA formats. You can also import nucleotide or amino acid sequences from an ASCII file of flexible format, and Vector NTI will automatically create the new database molecule and assign the sequence to the molecule.

Archives—A Tool For Sharing Database Information

Vector NTI lets you place your molecules, enzymes, oligonucleotides and gel markers into archives that can be transferred to another computer (Mac or PC) and read by Vector NTI. This allows you to share objects with colleagues, or to place them simultaneously on several computers. As molecules are archived, Vector NTI verifies the consistency of all information.

Important: The Vector NTI Database is completely independent of the Xpression NTI Database and each database has its own unique viewer. It is not possible to exchange objects between the Xpression NTI and Vector NTI applications.

Features and functions of Vector NTI Suite

Creating New Molecules

There are five different ways of creating new DNA/RNA and protein molecules in Vector NTI:

1. Importing from GenBank/GenPept, EMBL/SWISS-PROT and FASTA formats or from an ASCII file of flexible format. The sequence and Feature map are converted from the file, and the new molecule becomes part of the Vector NTI database.
2. Creating new molecules from user-defined nucleotide or amino acid sequences. These can be manually entered or pasted from the clipboard and the sequence entered as a new molecule.
3. Translating a coding region of an existing DNA or RNA molecule to create new protein molecules.
4. Construction of new DNA/RNA molecules from user-defined compatible component fragments from other molecules.
5. Design of new DNA/RNA molecules from components in a user-defined fragment list, using Vector NTI's built-in biological knowledge to design the recombination process.

All new molecules are integrated into the database and can participate in all further operations and analyses.

Creating New Enzymes, Oligos, and Gel Markers

Enzyme, oligonucleotide, and gel marker objects can be created "from scratch" using Vector NTI's editors or by importing them by a REBASE database.

Oligos and Primers

Vector NTI can design PCR primers, sequencing primers and hybridization probes and save them to the database for future use. Using parameters you have defined, Vector NTI can analyze those primers and probes or those you have defined yourself to determine the best ones for optimal experimental results.

Editing Database Objects

Full molecule editing capabilities are available in Vector NTI, either from Database Explorer or the Molecule Display window. In Molecule Display windows, you can edit molecules by inserting, deleting and replacing sequence fragments and features. You can also modify a molecule's display format and general data. When your editing is complete, you can save the molecule into the database to make the changes permanent.

Parent-Descendant connections

As you modify molecules, Vector NTI monitors and manages parent-descendant connections to insure data consistency through all the descendants (see Appendix D, page 725).

Generating Restriction Maps

Restriction maps of DNA/RNA molecules can be quickly generated in Vector NTI. For unsequenced molecule regions, you may enter the known positions of restriction sites. All the molecule descendants inherit these sites.

Molecule Display

Molecule display windows are Vector NTI's convenient graphical user interface (GUI) for inspecting and editing text description, graphical maps and analyses and sequences of DNA/RNA and protein molecules. They are also powerful and convenient graphical tools for selecting fragments for molecule construction and design and for preparing publication-quality images.

Molecule display windows consist of text, graphics and Sequence Panes.

Text Pane

A display window's Text Pane contains a general molecule description and folders describing a molecule's features, search results, cloning strategies and protocols, and biological data.

Graphics Pane

A molecule display window's Graphics Pane contains a graphically displayed or linear molecule map. In the Graphics Pane, you can visually select and "prepare" fragments for construction and design of new molecules, search for probes and primers, or for restriction maps.

All graphics objects are movable, resizable and editable.

Sequence Pane

A molecule display window's Sequence Pane contains the formatted nucleotide or amino acid sequence of a molecule.

- For DNA/RNA molecules, Vector NTI can show restriction sites, motifs, ORFs and functional signals and translated reading frames.
- For protein molecules, Vector NTI can display protein features together with the amino acid sequence.

You can print any molecule display window or you can save it to an external file or clipboard. From the clipboard, you can insert maps, sequences and molecule descriptions directly into word processing documents.

Creating Molecule Documents

You can save all the information from a molecule display window to a permanent storage by creating a Molecule Document file. Since the Molecule Document format is based on GenBank/GenPept formats, not only Vector NTI, but any program able to import GenBank/GenPept files can also import Vector NTI Molecule Documents.

Gel Display Windows

Gel Display windows can be created for different types of electrophoresis. The Gel Display window's Graphics Pane replicates the results of an actual gel.

Creating Gel Display Windows

To create a new Gel Display window you must define its parameters, as to electrophoresis and buffer types, electrophoresis conditions and display parameters.

You can modify the settings of a Gel Display window at any time after creation for better separation of the molecules' fragments.

Creating Gel Samples and Gel Markers

In Vector NTI, a gel lane may contain either a Gel Sample, or a Gel Marker, user-generated or from the database. Gel Samples are created by "cutting" database molecules with restriction enzymes. Gel Markers, sets of fragments of known lengths, can be loaded either from the database or can be user-generated.

Running the Gel

After a Gel Display window is created, and your samples and markers have been "added" to the "lanes," you can run a gel according to selected time increments or animation settings. You can color-code specific fragments or define "separation distances" or time lengths to better visualize fragment separation. Because of the simplicity of their generation, Gel Samples are created on the spot for short-term use, but are not stored in the Vector NTI database.

Align X

In AlignX, you can:

Perform multiple sequence alignments on either proteins or nucleic acids without reformatting between applications and computer platforms.

- In the alignment, link, localize and compare important domains in individual sequences
- Generate plots of similarity and sequence complexity

AlignX features include multicolor presentation, alignment editing, sequence comparison dot plots, phylogenetic trees, sequence and structure alignments, and similarity distribution plots. AlignX reads all standard text formats such as FASTA, GenBank, EMBL, SWISS-PROT, GenPept, and ASCII text.

BioPlot

BioPlot is a comprehensive set of protein and nucleic acid sequence analysis tools, offering over fifty different predefined protein scales linked with Feature maps and sequences.

BioPlot is formatted so that plots can be filed, overlapped, scaled, moved, and formatted to fit your presentation needs. Sequence regions can be highlighted by changing font face, size, and color and the plots linked to a molecule feature table. The use of a standard text file format in all programs eliminates the need to reformat data for use in any Vector NTI Suite modules.

ContigExpress

ContigExpress is a program for assembling many small fragments – either text sequences or chromatograms from automated sequencers – into longer, contiguous sequences. You can manage fragments and assemblies in an Explorer window, and open fragments and contigs in display windows for further study and manipulation. The multi-pane windows link sequences with their properties, chromatograms and graphic representations.

You can edit nucleic acid fragments directly with the chromatograms in full view. Changes are tracked and an edit history is maintained. The contigs generated are then saved and managed as GenBank, EMBL or FASTA files. No exporting or reformatting of proprietary file formats is necessary. Just drag and drop into Vector NTI to map and analyze.

Internet Tools

Vector NTI has Internet-friendly applications, allowing you to share your data and results of your work with your colleagues and to use remote bio-services for analyzing your data. Molecule Documents are automatically converted to HTML files, which you can then publish on a WWW site. You can analyze your data using Internet tools built into Vector NTI.

BLAST Search/BLAST Viewer

Vector NTI Suite provides you with a search engine for launching BLAST searches of the GenBank databases at the National Center for Biotechnology Information website. Results are listed in the BLAST Search dialog box from which they can be opened and reviewed in

BLAST Viewer, displaying a linear representation of aligned hit elements from the hit molecules.

Miscellaneous Tools

PubMed/Entrez Search, Citation Viewer and Citation Table of Database Explorer

These tools work in tandem to provide a practical and efficient way to search public databases for molecules or citations, retrieve them, complete with molecule files or abstracts that can be viewed and stored.

GCG Converter

GCG Converter is a tool in the Vector NTI Suite that converts sequences in GCG file formats so that they can be imported into the Suite.

Matrix Editor

Matrix Editor allows inspection of and editing of matrices. Matrices are used in two Vector NTI Suite applications, AlignX and AlignX Blocks. Matrices can be viewed in either program in the Alignment Setup dialog box, but editing can only take place in Matrix Editor.

AlignXBlocks

AlignX Blocks is a program for locating, analyzing and editing blocks of localized sequence similarity among multiple protein sequences and linking them into a composite multiple alignment.

3D-Mol

The 3D-Mol application of Vector NTI Suite allows you to visualize and manipulate 3-dimensional molecule structures described in a standard PDB (Protein Data Bank) file in a user-friendly environment.

CSV Oligo Files Importer

The CSV Files Importer utility allows you to import oligo lists into the Vector NTI database. The oligo list to be imported must be in one of three file formats for use with the CSV utility: Comma delimited format (.csv format), Tab delimited format or Semicolon delimited format.

Tools Manager

Vector NTI 7.0 is shipped with more than 20 tools providing connection to various Internet servers. You can add your own tools connecting Vector NTI to local or remote programs using the configuration mechanism described in this chapter. All of the Vector NTI tools are organized and managed using Tools Manager.

License Manager

Vector NTI 7.0 consists of three types of licenses: static, dynamic and trial license, with an additional demo mode for the purpose of demonstrating the Vector NTI Suite software. The various license types were developed to meet users needs. For example, users in different industrial, scientific and educational environments require different sharing and allotment privileges. These privileges are all administered through the License Manager.

Xpression NTI

Sophisticated techniques using microarrays allow scientists to characterize the mRNA expression level of thousands of genes simultaneously. A typical expression experiment generates thousands of data points, presenting serious challenges for storing and processing data. The desktop expression application, Xpression NTI, is a comprehensive program, independent of Vector NTI, designed to store and manage gene expression data. It provides the algorithms for processing and examining the data from single and multiple expression experiments.

Xpression NTI can merge primary expression run results. Filtering, sorting and clustering algorithms, parameterizations or profiling methods can be used to analyze and organize data. Huge volumes of quantitative data are displayed graphically to find inherent patterns of gene expression, both on a broad scale ("birds-eye" view) and fine scale (gene by gene).

Xpression NTI consists of two closely associated parts, the Expression Database Explorer and the Expression Viewer. The software is designed to work with gene expression data, managing the data by means of the Expression Database Explorer and analyzing and manipulating it in the Expression Viewer window. The Expression Viewer window also provides an interface for working with separate experiments not stored in the Expression Database. From the Expression Viewer window, experiments can either be saved to the Expression Database or to an independent file on the hard drive.

Important: The Xpression NTI Database is completely independent of the Vector NTI Database and each database has its own unique viewer. It is not possible to exchange objects between the Xpression NTI and Vector NTI applications.

Chapter 3 The Vector NTI and Xpression NTI User Interfaces

Introduction

This chapter introduces you to the Vector NTI and Xpression NTI user interfaces and, in specific cases, to Molecule Display windows, a powerful graphical tool for displaying and editing molecules. Included in this chapter are global operations used routinely in many of the Vector NTI Suite and Xpression NTI applications.

Vector NTI runs on both Windows and Macintosh platforms. Xpression NTI runs on a Windows platform. All window manipulations techniques standard for given platforms can be used with the programs, such as moving and sizing windows, menus, toolbars, selecting objects and so forth.

This chapter assumes that you are familiar with Molecule Display windows and with basic display window techniques, such as maximizing windows, changing the sizes of text and gel panes, switching between panes, etc.

Launching Vector NTI

To launch Vector NTI, double-click its icon in the program group or folder in which you installed the software. When the program is launched, the empty Vector NTI workspace is displayed. By default, the local Database Explorer window is displayed on top of it (you may turn this feature off). The Database Explorer is described in detail in Chapter 18.


Launching Xpression NTI

To launch Xpression NTI, double-click its icon in the program group or folder in which you installed the software. When program is launched, the Xpression NTI Database Explorer is displayed. The Xpression NTI Database Explorer is described in detail in Chapter 37.

Online Help

The Online Help has been written to assist you, the Vector NTI Suite or Xpression NTI user, in mastering the software.

In either of the applications, there are several avenues for receiving assistance through the Online Help:

- Press the **Help** button () , then click on any toolbar button, opening the associated help topic.
- Press the **Help** button, then click on any command from the menu bar, opening the associated help topic.
- Press F1 from an open dialog box, opening its associated help topic.
- Select **Help > Help** or **Using Index** from the menu bar of the application. In the Online Help that opens, you can browse through the Table of Contents or the Index, or launch a word search of the Online Help application.

If pressing F1 fails to open an Online Help topic, select **Help > Help Topics**, opening Online Help. Proceed with a Browse through the Table of Contents or Index or do a word search. Your topic may be in the Help files, but inadvertently not linked to its associated dialog box.

Topics may be titled by their function rather than the dialog box name. For example, the New Molecule dialog box associated topic is named "Creating a New Molecule."

The Application Workspace

A typical Vector NTI or Xpression NTI display window can include:

- Text information, including object descriptions, features, recombinant strategies and analysis results
- Graphical features of a molecule, a gel, or a contig assembly
- Analytical features of a molecule
- A pairwise or multiple alignment
- Appropriate sequences

From database or display windows:

- Local databases can be searched
- Public domain database searches can be launched through the Internet
- Text descriptions, graphical maps and sequences are edited
- Graphical representations may be formatted
- DNA/RNA and protein molecules and search results are stored in a database
- Preparation of publication-quality figures is supported.

Important: The Xpression NTI Database is completely independent of the Vector NTI Database, and each database has its own unique viewer. It is not possible to exchange objects between the Xpression NTI and Vector NTI applications.

Viewer Display Window Description

The display window title bar shows the name of the molecule on display or the name of the folder being displayed.

Every display window has a menu bar. Options in the submenus vary according to the specific application being used.

Shortcut menus associated with folders, molecules or objects from which they are opened provide many options for operations with those items. To view a shortcut menu, right-click while the cursor is hovering over the object.

A shortcut menu is opened with a right click with the cursor paused over the related item.

All display windows contain two or more toolbars and variants relative to the active display pane.

- The Main Toolbar contains tools for major program functions, such as opening, saving, and printing Display windows, undoing and re-doing actions, launching major subsections of the program such as the Database Explorer, etc.
- The Window Toolbar has several different variants according to the activated pane of the topmost display window.

Many menu and toolbar options are consistent throughout the Vector NTI Suite; many are also consistent in Xpression NTI. Toolbar commands are often duplicated on the menu bar and shortcut menus, offering several means of accomplishing the same objective.

For information on all toolbars specific to Vector NTI and Xpression NTI applications, refer to Chapter 4. Toolbar buttons pertaining to operations and objects being described throughout the manual are displayed as needed.

When you launch Xpression NTI, the Xpression NTI Database Explorer opens. Expression Data Viewer can only be launched from the Xpression NTI Database Explorer. For details, see Chapter 3.

When you launch Vector NTI, the empty workspace is displayed, super-imposed by the Database Explorer window. Once you minimize Database Explorer and open a molecule in the Molecule display window, the following features can be viewed. See Fig. 3.1.

Viewer Panes

Most display windows in the Vector NTI Suite are divided into four sections, or panes:

- A Text Pane holds folders containing text descriptions and analyses results for a molecule shown in the Display window.
- A Graphics Pane displays a graphical depiction of a molecule. Features, restriction sites, motifs, etc. are shown by labeled symbols.
- An Analysis Pane allows certain types of DNA and protein sequence analysis properties to be performed and the results to be viewed as linear graphics.
- A Sequence Pane shows a nucleotide or amino acid sequence and functional features of a molecule.

Variations of this will be explained in appropriate chapters.

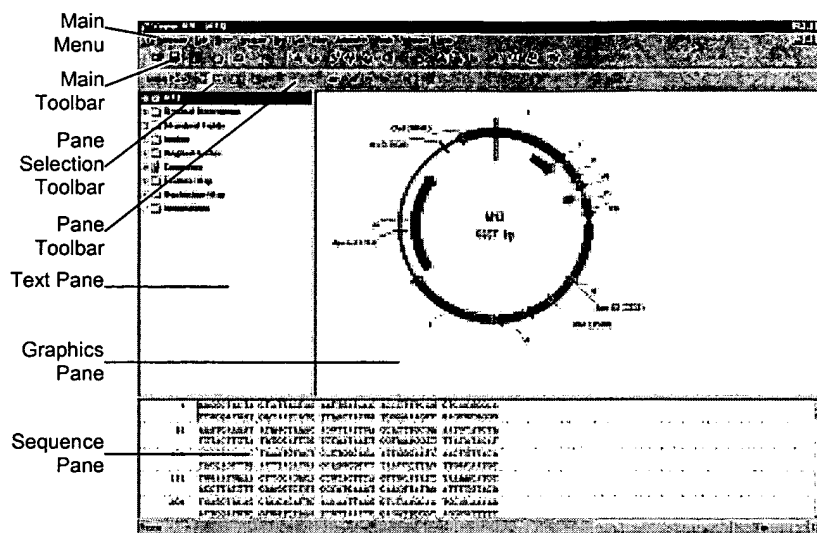


Fig. 3. 1 Molecule display window

Viewer Window Operations

The following global operations for viewer windows apply to the Vector NTI Suite applications and to Xpression Data Viewer. For details about operations specific to each application, refer to the appropriate chapters.

Activating a Pane

Click anywhere in a pane to activate it or click on the pane buttons appearing at the left of the lower toolbar.

Note: Clicking in a pane sometimes selects an entire molecule. To avoid this, use the toolbar buttons to activate the pane.

Re-sizing of the Display Panes

Split bars separate the text, graphics, and Sequence Panes. Re-size the panes by dragging the split bar that divides the panes. Position the cursor on the split bar until it changes to a two-headed arrow. LEFT-CLICK and drag to reposition the split bar.

Scrolling the Text Pane

Scroll bars appear when the contents of a pane exceed the viewing area. Move the scroll bars vertically or horizontally to reposition the pane contents for easier viewing. If the active pane is a Text Pane, it can also be scrolled with the arrow keys on the keyboard.

Layout Options

- Select **View > Maximize Pane** to expand the size of an activated pane to full screen size. Select **View > Restore Layout** or press the **ESC** button to return to the original pane layout.

- Select **View > Edit Pane Layout** to open the Pane Manager for modifying the arrangement of the panes in the workspace. For more details, refer to page 20.

Viewer Display Panes

Text Pane

Text Pane folders contain descriptions of molecules and other objects, their characteristics and features, search results, methods and protocols.

Opening and Closing Folders

To open or close a folder, click the small square located to the left of the folder, double-click on the folder or line, select **View > Toggle Folder** or select the folder and press ENTER.

Opening or closing a folder does not affect its state.




Expanding and Collapsing Folders

Opening of a folder simultaneously with some or all of its subfolders is called expanding that folder. Closing a folder simultaneously with some or all of its subfolders is called collapsing that folder. A folder and its subfolders are called a branch.

The View menu (and the shortcut menu) has commands for expanding and collapsing folders and subfolders in various combinations:

Command	Result
Expand One Level	Opens the focused folder; makes sure all subfolders are closed
Expand Branch	Opens the focused folder and all of its subfolders
Expand All	Opens every folder and subfolder in the text pane
Collapse Branch	Closes the focused folder and all of its subfolders
Collapse All	Closes every folder and subfolder in the text pane
Collapse Around	Closes all folders and subfolders, except any highlighted folders and their parents


Table 3. 1 View menu commands for expanding and collapsing folders and subfolders

Corresponding toolbar buttons: Expand Folder () , Expand Branch () and Collapse Branch ()

Linking the Display window panes

Linking the panes of a display window gives the Text Pane control over the information displayed in the Graphics and Sequence Panes. When linked, information from any open folder in the Text Pane is displayed in the Graphics and Sequence Panes, while information in closed folders is not displayed. *Certain minimum information is always displayed,*


however: a molecule name and length in a Graphics Pane and the nucleotide or amino acid sequence in a Sequence Pane.

To link panes, activate the Text Pane and press the **Link Panes** button () on the Window toolbar.

Linking panes reduces clutter, displaying only those objects that are important to your current task and lets you display symbols hidden by Vector NTI's Standard Arrangement of graphics objects. The Standard Arrangement of DNA/RNA molecules' graphics displays a maximum of 70 restriction sites plus 70 motifs, displayed to show as many different enzymes and motifs as possible.


You can override this display by linking the panes and opening the text folders for the only signals whose every occurrence you want to display.

Use the linked panes to create attractive publication-quality figures without switching to the Picture Editing mode and editing the picture manually. With the panes linked, close all folders, then open the folders of the signals you want to display, and then click the

Standard Arrangement button (). Symbols and labels will be redistributed to take maximum advantage of available space.

Finding Objects on Graphics and Sequence Panes


Some objects, described in the Text Pane, are also displayed in the graphics and Sequence Panes. To find the position of the object, select its line in the Text Pane and press the **Find**


button () in the window toolbar, select **Edit > Find** or press CTRL+ F. The object will be selected on the Sequence and Graphics Panes. *The Find command is disabled if the selected line does not describe the object, displayed in two other panes.*

Note: Vector NTI finds only single objects like a restriction site, not multiple objects like all restriction sites for a certain REN.


Graphics Pane


Zooming the Graphical Map In and Out


To enlarge a graphical map, activate the Graphics Pane and click the **Zoom In** button () on the Window Toolbar or choose **View > Zoom In**. The keyboard shortcuts are] and SHIFT +]. To zoom in by a small increments, SHIFT + CLICK the **Zoom In** button.


To zoom out from a graphical map (reduce it by 50%), click the **Zoom Out** button () on the Window toolbar or choose **View > Zoom Out**. The keyboard shortcuts are [and SHIFT + [. To zoom out by a small increments, hold down the SHIFT key and click the **Zoom Out** button.

If the current display is linear, change the horizontal zoom factor only using CTRL + CLICK on the **Zoom In** or **Zoom Out** button. Changing only the horizontal zoom factor changes the internal aspect ratio of the graphical display; this ratio is saved when you save Display Setup and can be applied to other Display windows as well.

To fit a graphical map to the current size of the Graphics Pane, click the **Fit to Window** button () on the Window toolbar or choose the equivalent command in the View menu.

To zoom into a selection on the graphics map, making the selection the current size of the Graphics Pane, click the **Fit Selection to Window** button () on the toolbar or choose the equivalent command in the View menu. In some display windows, you can select the

View Selection button (). To return to the original view, select **View > View Whole Molecule**.

One button is specific to the Gel Display window: Press the **True-Scale View** button () to display the gel in an approximation of true scale. When you select this display option, Vector NTI looks up the pixel size (dot pitch) of your monitor and displays the gel based on that value.

Sequence Pane

Formatting Sequence Text

To format sequence text, select it by click + dragging the mouse across the text. Because the Sequence Pane is active, you can use any of the following buttons or keystrokes to format the text.






<i>Button</i>	<i>Keystroke</i>	<i>Description</i>
	CTRL-B	Set or clear bold character(s) attribute
	CTRL-I	Set or clear italic character(s) attribute
	CTRL-U	Set or clear underline character(s) attribute
Font style drop-down menu	--	Select font name for character(s)
Font size drop-down menu	--	Select font size for character(s)
	--	Select font color for character(s)
	--	Select background color for character(s)


Table 3. 2 *Formatting sequence text*

Miscellaneous Interface Features

Selection Techniques

To select text in a Molecule Viewer display window's Text Pane, click on the line you wish to select. To select multiple lines in a Sequence or Text Pane, press the mouse button and drag across the lines you wish to select.

To make a selection in a display window's Graphics Pane, choose **View > Edit Picture**

with an active Graphics Pane or press the **Edit Picture** button () to enter the Picture Editing mode and click on any object or label to select it. To select more than one object, click on each object in turn while holding down the SHIFT key.

To select text in a Sequence Pane, click and drag the mouse across the text.

You can select an entire pane by making that pane the active pane and choosing **Edit > Select All**.

Copying Display Window Text, Graphics or Sequences Image To The Clipboard

Copying in Vector NTI and Xpression NTI can take several different forms:

Edit > Camera or Camera Button ()

The Camera feature copies the graphical representation, or "takes a picture" of the selection or activated pane contents. The contents are copied to the clipboard from where they are pasted into a new page or slide in other running applications.

Activate a pane or make a selection, then press the **Camera** button or choose **Edit > Camera**. In the dialog box that opens, choose the range and destination for the copied pane.

- **Range** - Specify the range of data to be copied. The selection option is unavailable if a selection is not defined in the active pane.
- **Copy To** - Specify the destination of the copy operation. If the File option is selected, the Copy To dialog box opens where the name of the output file can be specified.

Note: If nothing is selected, the entire pane is copied. The format of this dialog box may vary slightly in different applications.

Copying Sequence or Alignment Pane Data

In copying the contents of a Sequence or Alignment Pane to the clipboard, the Camera dialog box offers two formats for the copy:

- **Metafile picture:** only the snapshot of the Sequence or Alignment Pane will be copied to the Clipboard
- **Text:** you can further specify to print either the entire alignment or only the selected part of it (if there is selection at the moment). Sequences may or may not be wrapped. It can then be pasted into any text processor.

Note: A double-stranded sequence copied from Vector NTI becomes single-stranded when it is pasted.)

The Clipboard stores Vector NTI text, sequence, alignment and graphical representations in both RTF (Rich Text Format preferred by word processors) and in plain text format (for e-mail and other applications preferring plain text).

Note: The RTF representation might not have exactly the same look as the sequence in the Sequence Pane due to limitations of RTF.

Vector NTI graphics are stored as Windows Metafile (Windows).

Edit > Copy or Copy Button

This copy feature transfers actual sequence and annotations, the molecule file, rather than just the graphical representation. Molecules copied this way can be shared and used in other Vector NTI Suite applications.

Text Pane data is copied a plain multilane text. Sequence data is copied as a plain text string. All sequence formats recognized by Vector NTI can be pasted from the Clipboard. Graphics Pane data is copied as a Windows Metafile.

Make the selection, then press the **Copy** button or select **Edit > Copy**. The copied data is copied clipboard or file in text format. Preserves formatting and enables easy data exchange with other applications.

Copying Raw Sequence

To copy real sequence data to the Clipboard, select a sequence region and choose Edit Copy. The sequence is copied in the plain text format without formatting or line numbering. This “raw” format is suitable for pasting into other molecular biology software including programs in the Vector NTI Suite. The shortcut for the copy operation is Ctrl + C.

Edit > Copy To Command


This copy command saves a whole molecule or selection of a molecule to various file formats, including saving a DNA/RNA sequence to a protein translation. If the whole sequence is chosen, you have a choice of saving to GenBank, EMBL, FASTA, plain sequence or amino acid translation (for DNA molecules). For a selection, the file format choices are FASTA, plain sequence or amino acid translation (for DNA selections).

Open a molecule, make the selection, if desired, and choose **Edit > Copy To** from the pull down menu. Choose the desired file type and storage location. Choose whole molecule or selection.

The Edit > Copy To command is implemented in Vector NTI and the Vector NTI Database Explorer.

Printing from Display Windows

Display windows are printed one pane at a time. To print a pane, activate it, then press the

Print button () on the main toolbar or choose **File > Print**.

Note: Print copies are similar to the screen display, so adjust the picture on screen before printing.

Page Setup


Text and Sequence Panes are printed in a standard format. For the text and sequence, you can change the content of what is printed, but not the layout. If a Text Pane is activated, any open folder is printed.

Graphical maps can be printed out at any size and in various positions on the page.

To change the size and position of a graphical map's printout, select **File > Page Setup**. In the dialog box, set the margins (in inches) for your printout. Select **File > Print Setup** to specify the page orientation and size. Click **OK** to print.

Print Preview

To see a preview on your screen of what the printout will look like, choose **Molecule >**

Print Preview or press the **Print Preview** button ()

If you want to print just a selection, use the camera option. Select the region, click the camera on the Main Toolbar, paste the copied selection to a new application (such as word processing software) and print from there. (See next section.)

Exporting Molecule Data

BioPlot allows simple export of a molecule as GenBank (GenPept for proteins), EMBL (SWISS-PROT for proteins), FASTA, or as text sequence. To export a molecule to an external file, choose **File > Save As** and select file type in the standard File save dialog box.

Pane Manager

In most components of the Vector NTI Suite, you can modify the layout of the display panes. To open the Panes Layout Editor, sometimes called Pane Manager, where you can modify display pane layout, select **View > Change Panes Layout**.

The main elements of the Pane Manager editor dialog box (Fig. 3.2) are the hierarchy layout tree, the preview panel, and the drop-down menu (with a list of predefined layouts).

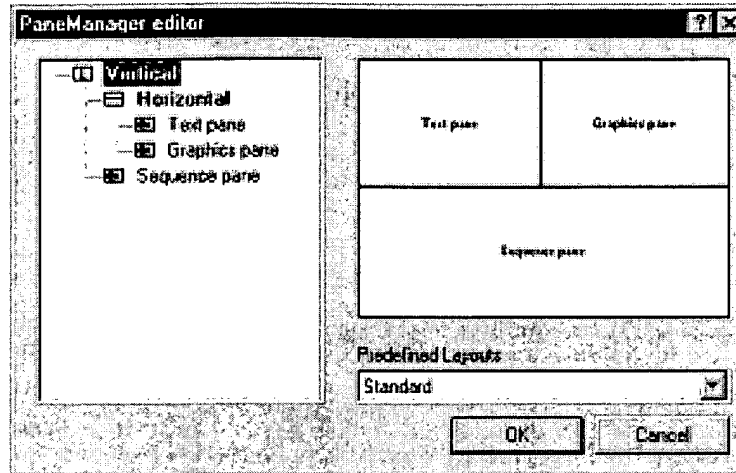


Fig. 3. 2 Pane Manager editor dialog box

The hierarchy layout tree represents the current layout (always the last settings used), while the node (Vertical, Horizontal, or Tabular) describes the relationship between the panes.

Options for modifying the pane layout appear on the shortcut menu, opened from the Vertical, Horizontal and Tabular nodes or from the Text, Graph or Sequence Pane leaves. Shortcut menu options are as follows:

- **Change Layout** - For Vertical, Horizontal, or Tabular nodes, this operation changes the relationship between underlying leaves (designated with green icons).
- **Dismiss** - This operation does not affect the root node. It removes the selected node, and shifts all of the underlying leaves to control of the root node. For example, if you dismissed **Horizontal** in the above graphic, the Text, Graph, and Sequence Panes would all be displayed vertically.
- **New sublayout** - This adds a new node in the designated area.
- **Up** - This will move the node or leaf up in the tree.
- **Down** - This will move the node or leaf down in the tree.

You can also change the parent node for any node or leaf. All you have to do is just drag the node or leaf and drop it to new parent node.

The Predefined Layouts drop-down menu contains a list of predefined pane layouts.

The preview panel of the editor shows the layout for the current state of the hierarchy tree and is redrawn immediately when you change the layout. This panel is immediately redrawn after the state of the hierarchy tree is changed. The Info Pane is generally known as the Text Pane in most Vector NTI applications.

Press the **OK** button after editing the layouts to apply your changes to the real panes or press **CANCEL** to discard your changes.

Notes About Pane Manager:

- You cannot add or delete a pane leaf. You can only move these leaves and configure their output.
- The Tabular selection places tabs at the bottom of each pane in the window.
- The Predefined option Standard returns the layout to the original.

Chapter 4 Vector NTI Suite and Xpression NTI Toolbars

Introduction












This chapter gives a brief pictorial overview of toolbar buttons and descriptions of their functions as they are used in Vector NTI Suite and Xpression NTI software.

The buttons described in this chapter are divided according to the application. Within each module, there is a *Main Toolbar* section containing buttons used for major program functions, such as opening and saving projects, adding molecules, etc. In most modules there is a *Pane Selection Toolbar* with buttons used to switch between the active panes. A *Pane Toolbar* containing buttons for manipulating the active display window follows the *Pane Selection Toolbar*.

A brief description of the functions of each tool on each toolbar is presented below. Many toolbar commands can be launched from the menu bar, from a shortcut menu or from keystrokes.

Molecule Display/Gel Display windows

Molecule Display/Gel Display Main Toolbar

<i>Button</i>	<i>Description</i>
	open an existing project
	save the current project
	create a display window for a new gel
	send the active window as an Email document
	copy data to clipboard
	undo the previous editing action
	redo the previous editing action
	display a print preview
	print the active pane
	open the local database explorer
	connect to a shared database












Button	Description
	prepare the local and shared database explorer window for data exchange
	add a new fragment to the goal list
	add selection to the oligo list
	add selected fragments to the to gel sample list
	open the goal molecule definition list
	open the oligo list
	open gel sample list
	execute the last tool
	access information about Vector NTI
	open Vector NTI World Wide Web home page
	displays online help

Table 4. 1 Molecule Display/Gel Display Main toolbar

Molecule Display/Gel Display Pane Selection Toolbar







Button	Description
	switch to the Text Pane
	switch to the Graphics Pane
	switch to the Gel Graphics Pane
	switch to the Sequence Pane

Table 4. 2 Molecule Display/Gel Display Pane Selection toolbar

Molecule Display/Gel Display Text Pane Toolbar

Button	Description
	display setup
	view molecule fragment



















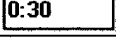
Button	Description
	create a new gel sample
	add a gel marker from the database to a new lane
	add selected fragments to the gel sample list
	add a new lane containing the gel sample list fragment
	find the selected signal in other panes
	link panes
	expand current folder
	expand current folder and all subfolders
	collapse current folder and all subfolders
	delete a molecule from the project
	add selection to the oligo list

Table 4. 3 Molecule Display/Gel Display Text Pane toolbar

Molecule Display/Gel Display Graphics and Gel Graphics Pane Toolbar

Button	Description
	display setup
	view molecule fragment
	create a new gel sample
	add a gel marker from the database to a new lane
	add selected fragments to the gel sample list
	add a new lane containing the gel sample list fragment
	reverse the gel one increment of time per click.
	track gel run time




















Button	Description
	advance the gel one increment of time per click.
	animate gel run
	calculate the time for separation of selected fragments
	add feature
	graphics display setup
	search for nucleotide sequence
	circular display
	linear display
	increase graphs' horizontal scale
	decrease graphs' horizontal scale
	scale graphs to fit the pane window
	true scale display of gel
	standard arrangement
	translate currently selected nucleotides in direct strand
	translate currently selected nucleotides in direct complementary strand
	edit picture
	add annotation

Table 4. 4 Molecule Display/Gel Display Graphics and Gel Graphics Pane toolbar

Molecule Display Sequence Pane Toolbar

Button	Description
	display setup
	view molecule fragment



















Button	Description
	add feature
	search for nucleotide sequence
	cut out current selection while copying it to the clipboard
	copy current selection to the clipboard
	paste data from the clipboard
	toggle one/two strands mode
	translate currently selected nucleotides in direct strand
	translate currently selected nucleotides in direct complementary strand
	clear all translations currently shown
	toggle the bold mode for the selected character(s)
	toggle the italic mode for the selected character(s)
	toggle the underline mode for the selected character(s)
	choose the font size for the selected character(s)
	choose the font name for the selected character(s)
	choose the background color for the selected character(s)
	choose the font color for the selected symbols

Table 4. 5 Molecule Display Sequence Pane toolbar

AlignX Toolbars**AlignX Main Toolbar**

Button	Description
	open an existing project
	saves the current project











Button	Description
	adds a molecule(s) to the project
	multiple alignment
	multiple alignment using profile
	add molecule(s) to existing alignment
	options for pairwise, multiple, profile alignments
	open Dot Matrix window
	display a print preview
	print active pane
	copy data to clipboard
	displays online help

Table 4. 6 AlignX Main toolbar

AlignX Pane Selection Toolbar










Button	Description
	switch to the Text Pane
	switch to the PhylogeneticTree Pane
	switch to the Graphics Pane
	switch to the Alignment Pane



Table 4. 7 AlignX Pane Selection toolbar

AlignX Text Pane Toolbar










Button	Description
	expand current folder
	expand current folder and all subfolders
	collapse current folder and all subfolders

Button	Description
	delete a molecule from the project
	find the selected signal in other panes



*Table 4. 8 AlignX Text Pane toolbar***AlignX Phylogenetic Tree Pane Toolbar**

Button	Description
	remove a molecule from alignment
	save phylogenetic tree to .ph file

*Table 4. 9 AlignX Phylogenetic Tree Pane toolbar***AlignX Graphics Pane Toolbar**

Button	Description
	increase graphs' horizontal scale
	decrease graphs' horizontal scale
	scale graphs to fit the pane window
	zoom graphs to selection
	toggle the vertical axis
	toggle the legends
	toggle the legends position
	open the analysis dialog box
	open plot setup dialog box

*Table 4. 10 AlignX Graphics Pane toolbar***AlignX Alignment Pane Toolbar**

Button	Description
	open edit alignment dialog
	remove a molecule from alignment


Button	Description
	open alignment display setup dialog

Table 4. 11 AlignX Alignment Pane toolbar

Dot Matrix Toolbar, AlignX

The Dot Matrix application is accessible within AlignX. Window.

The molecules' selection toolbar has 2 drop-down menu controls to select the pair of molecules for a Dot Matrix.

Dot Matrix Main Toolbar












Button	Description
	open Dot Matrix setup
	undo previous zoom
	redo previously undone zoom
	maintain isotropic scaling
	show grids on zoomed matrix
	display a print preview
	print active pane
	copy data to clipboard
	display online help




Table 4. 12 Dot Matrix Main toolbar

Similarity Table Toolbar, AlignX











The Similarity Table application is accessible from the Alignment drop down menu in the AlignX window.

Similarity Table Main Toolbar

Button	Description
	show similarity values on the top section of the table
	show divergence values on the top section of the table

Button	Description
	show similarity values on the top and divergence values on the bottom
	print the current table
	copy the current table to the clipboard

*Table 4. 13 Similarity Table Main toolbar***BioPlot Toolbars****BioPlot Main Toolbar**

Button	Description
	open an existing molecule
	save a BioPlot project
	copy data to the clipboard
	paste data from the clipboard
	open the analyzer setup dialog
	open the analyses list dialog
	display a print preview for the active pane
	print the active pane
	open the camera dialog
	displays online help

*Table 4. 14 BioPlot Main toolbar***BioPlot Pane Selection Toolbar**




Button	Description
	switch to the Text Pane
	switch to the Plot Pane
	switch to the Sequence Pane

Table 4. 15 BioPlot Pane Selection toolbar

BioPlot Text Pane Toolbar





Button	Description
	expand the current folder
	expand the current folder and all subfolders
	collapse the current folder and all subfolders
	find the selected feature in the other panes

Table 4. 16 BioPlot Text Pane toolbar

BioPlot Plot Pane














Button	Description
	toggle the vertical axis
	toggle the legends
	toggle the legends position
	open the plot setup dialog
	increase the plots horizontal scale
	decrease the plots horizontal scale
	scale the plots to fit the pane window
	zoom the plots to selection
	switch to the edit layout mode
	switch to the overlapped mode
	bring the active plot(s) to the top
	send the active plot(s) to the bottom

Table 4. 17 BioPlot Plot Pane

BioPlot Sequence Pane

Button	Description
	toggle one/two strands mode

Button	Description
	translate currently selected nucleotides in direct strand
	translate currently selected nucleotides in direct complementary strand
	clear all translations currently shown
	switch to the format mode
	toggle the bold mode for the selected sequence
	toggle the italic mode for the selected sequence
	toggle the underline mode for the selected sequence
	choose the font name for the selected sequence
	choose the font size for the selected sequence
	choose the background color for the selected sequence
	choose the font color for the selected sequence

Table 4. 18 BioPlot Sequence Pane

ContigExpress Toolbars

Project Explorer Toolbar

Button	Description
	locate and open an existing ContigExpress Project
	save a ContigExpress Project to file
	view the Tree pane in History mode
	view the Tree pane in Content mode
	assemble the currently selected fragments
	dismiss an assembly
	open the assembly setup options




















Button	Description
	copy the currently selected item(s) to clipboard
	paste data from the clipboard
	rename currently selected item
	delete currently selected item(s) from the project
	displays the properties of the selected item(s)
	view the List pane in Large Icons mode
	view the List pane in Small Icons mode
	view the List pane in List mode
	view the List pane in Details mode
	enable the Show Contigs filter in List pane
	enable the Expand Contigs filter in List pane
	enable the Show Unassembled Fragments filter in List pane
	enable the Show Other Fragments filter in List pane
	invoke the Camera for the List pane
	enables easy access to specified topics

Table 4. 19 Project Explorer toolbar

ContigExpress-Fragment Window

CE-Fragment Window Main Toolbar

Button	Description
	save changes in active window back to project
	cut out current selection while copying it to the clipboard
	copy current selection to the clipboard
	paste data from the clipboard








Button	Description
	undo the previous editing action
	redo the previous editing action
	open camera dialog
	open viewing options dialog
	display a print preview for active pane
	print the active pane contents
	display online help

Table 4. 20 CE-Fragment Window Main toolbar

CE-Fragment Window Pane Selection Toolbar




Button	Description
	switch to the Text Pane
	switch to the Sequence Pane
	switch to the Chromatography Pane

Table 4. 21 CE-Fragment Window Pane Selection toolbar

CE-Fragment Window Text Pane Toolbar






Button	Description
	Expand the current folder
	Expand the current folder and all subfolders
	Collapse the current folder and all subfolders
	Find the selected feature in the other panes

Table 4. 22 CE-Fragment Window Text Pane toolbar

CE-Fragment Window Sequence Pane Toolbar

Button	Description
	search for nucleotide sequence




















Button	Description
	find previous ambiguous nucleotide
	find next ambiguous nucleotide
	set line width (number of nucleotides shown in one line)
	toggle one/two strands mode
	translate currently selected nucleotides in direct strand
	translate currently selected nucleotides in complementary strand
	toggle one/three-letter protein translation codes
	toggle shows ORFs for selected sequence
	toggle shows/hides peaks from deleted bases
	clear all translations currently shown
	switch to format mode
	choose the font name for the selected sequence
	choose the font size for the selected sequence
	toggle the bold mode for the selected sequence
	toggle the italic mode for the selected sequence
	choose the background color for the selected sequence
	choose the font color for the selected sequence

Table 4. 23 CE-Fragment Window Sequence Pane toolbar

CE-Fragment Window Chromatogram Pane Toolbar

Button	Description
	search for nucleotide sequence
	find previous ambiguous nucleotide



















Button	Description
	find next ambiguous nucleotide
	zoom in chromatogram graph
	zoom out chromatogram graph
	show/hide A trace on the chromatogram toggle
	show/hide C trace on the chromatogram toggle
	show/hide G trace on the chromatogram toggle
	show/hide T trace on the chromatogram toggle
	show/hide peaks for deleted bases toggle button

Table 4. 24 CE-Fragment Window Chromatogram Pane toolbar

ContigExpress- Contig Window

CE-Contig Window Main Toolbar

Button	Description
	Save changes in active window back to project
	cut out current selection while copying it to the clipboard
	copy current selection to the clipboard
	paste data from the clipboard
	undo the previous editing action
	redo the previous editing action
	open camera dialog
	open viewing options dialog
	display a print preview for active pane
	print the active pane contents


Button	Description
	display online help

Table 4. 25 CE-Contig Window Main toolbar

CE-Contig Window Pane Selection Toolbar




Button	Description
	switch to the Text Pane
	switch to the Graphics Pane
	switch to the Alignment Pane

Table 4. 26 CE-Contig Window Pane Selection toolbar

CE-Contig Window Text Pane Toolbar












Button	Description
	expand the current folder
	expand the current folder and all subfolders
	collapse the current folder and all subfolders
	find the selected feature in the other panes
	assemble fragments selected in the Text Pane
	assemble all fragments except the selected fragments

Table 4. 27 CE-Contig Window Text Pane toolbar

CE-Contig Window Graphics Pane Toolbar

Button	Description
	move fragment left
	move fragment
	move fragment right
	reverse complement fragment
	show ORFs on the graphics display




Button	Description
	zoom in contig graph
	zoom out contig graph
	fit the graph to screen

Table 4. 28 CE-Contig Window Graphics Pane toolbar

CE-Contig Window Alignment Pane Toolbar














Button	Description
	move fragment left
	move fragment up and down
	move fragment right
	reverse complement fragment
	move fragment back to the left
	move fragment back to the right
	show/hide chromatogram in the highlighted fragment
	translate consensus sequence in the first frame
	translate consensus sequence in the second frame
	translate consensus sequence in the third frame
	find sequence fragment
	find a previous ambiguous symbol in the consensus
	find a next ambiguous symbol in the consensus

Table 4. 29 CE-Contig Window Alignment Pane toolbar

AlignX Blocks Toolbars

AlignX Blocks Main Toolbar


















Button	Description
	open file or workspace
	save current file
	add file(s) to the project
	search for blocks in highlighted sequences
	AlignX Blocks parameter setup
	link selected area
	unlink selected area
	undo link operation
	redo link operation
	print preview
	print current page
	copy an item to a file or to the clipboard
	display online help






Table 4. 30 AlignX Blocks Main toolbar

AlignX Blocks Pane Selection


Button	Description
	switch to the Text Pane
	switch to Block List Pane

Button	Description
	switch to Schematic Pane
	switch to Alignment Pane


*Table 4. 31 AlignX Blocks Pane Selection***AlignX Blocks Text Pane**

Button	Description
	expand folder
	expand branch
	collapse folder
	delete folder from project (see below)
	find signals (see below)

*Table 4. 32 AlignX Blocks Text Pane***AlignX Blocks Block List Pane**

Button	Description
	edit selected block

*Table 4. 33 AlignX Blocks Block List Pane***AlignX Blocks Schematic Pane**

Button	Description
	define new block

*Table 4. 34 AlignX Blocks Schematic Pane***AlignX Blocks Alignment Pane**



Button	Description
	AlignX Blocks parameter setup
	define new block

Table 4. 35 AlignX Blocks Alignment Pane

3D Mol Toolbars

3D-Mol Main Toolbar







<i>Button</i>	<i>Description</i>
	load file
	save file
	print preview
	save as function
	copy an item to a file or the clipboard
	display online help

Table 4. 36 3D-Mol Main Toolbar

3D-Mol Pane Selection Toolbar









<i>Button</i>	<i>Description</i>
	switch to Text Pane
	switch to the 3D Pane
	switch to Sequence Pane

Table 4. 37 3D-Mol Pane Selection Toolbar

3D-Mol Text Pane Toolbar

<i>Button</i>	<i>Description</i>
	expand folder
	expand branch
	collapse branch
	mark selection feature(s) to be shown in the 3-D structure
	unmark selection feature(s) shown in the 3-D structure




Button	Description
	clear all marked regions
	show marked atoms
	hide marked atoms

Table 4. 38 3D-Mol Text Pane toolbar

3D-Mol Graphics Pane Toolbar
















Button	Description
	maximize Graphics Pane to fit the screen. Press Esc to return back to three pane mode
	scale the 3D molecule to fit the pane window
	zoom the 3D molecule to selection
	invert selection
	unmark all
	show marked atoms
	hide marked atoms
	show all atoms
	hide marked atoms
	switch to movement mode
	measure distance between two atoms
	measure valent angle between three atoms
	measure torsion (dihedral) angle between four atoms
	remove measurement marks
	colors marked atoms to make them more visible

Table 4. 39 3D-Mol Graphics Pane toolbar

3D-Mol Sequence and Alignment Panes Toolbar






Button	Description
	mark selection
	unmark selection
	mark all
	show marked atoms
	hide marked atoms

Table 4. 40 3D-Mol Sequence and Alignment Panes toolbar

Citation Viewer Toolbars

A brief description of the functions of the main toolbar is presented below:

Citation Viewer Main Toolbar











Button	Description
	back to previous document
	go to next document
	open an existing document
	save the active document with a new name
	copy data to the clipboard
	copy an item to a file or the clipboard
	print the active document
	display the full page
	bring up database explorer window
	display online help

Table 4. 41 Citation Viewer Main toolbar

PubMed/Entrez Search Toolbars

A brief description of the functions of the main toolbar is presented below:

PubMed/Entrez Search Main Toolbar

















<i>Button</i>	<i>Description</i>
	back to previous document
	go to next document
	terminate query process
	open a new window for a new search
	open an existing parameter file
	save parameter
	copy data to the clipboard
	undo a performed action
	display online help
	perform a search using the submitted conditions

Table 4. 42 PubMed/Entrez Search Main Toolbar

BLAST Search Toolbars

A brief description of the functions of each tool on the main toolbar, pane selection toolbar, and pane toolbars are presented below:

BLAST Search Main Toolbar

<i>Button</i>	<i>Description</i>
	open a new window for a new search
	open an existing parameter file
	save parameter
	back to previous document
	go to next document
	options for specifying technical settings


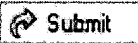
Button	Description
	display online help
	perform a search using the submitted conditions

Table 4. 43 BLAST Search Main toolbar

BLAST Viewer Toolbars

A brief description of the functions of each tool on the main toolbar, pane selection toolbar, and pane toolbars are presented below:

BLAST Viewer Main Toolbar















Button	Description
	back to previous document
	go to next document
	open an existing document
	save the active document with a new name
	copy an item to a file or the clipboard
	print the active document
	display the full page
	bring up database explorer window
	display online help




Table 4. 44 BLAST Viewer Main toolbar

BLAST Viewer Pane Selection Toolbar





Button	Description
	switch to the Text Pane
	switch to the Sequence Profile/Hit Distribution Pane
	switch to the Query-Hit Alignment Map Pane

Button	Description
	switch to the Hit Map Pane
	switch to the Alignment Pane

*Table 4. 45 BLAST Viewer Pane Selection toolbar***BLAST Viewer Text Pane Toolbar**

Button	Description
	expand current folder
	expand current folder and all subfolders
	collapse current folder and all subfolders

*Table 4. 46 BLAST Viewer Text Pane toolbar***BLAST Viewer Sequence Profile/Hit Distribution Pane Toolbar**

Button	Description
	increase graphs' horizontal scale
	decrease graphs' horizontal scale
	scale graphs to fit the pane window
	options for specifying technical settings

*Table 4. 47 BLAST Viewer Sequence Profile/Hit Distribution Pane toolbar***BLAST Viewer Hit Map Pane Toolbar**





Button	Description
	increase graphs' horizontal scale
	decrease graphs' horizontal scale
	scale graphs to fit the pane window
	options for specifying technical settings

Table 4. 48 BLAST Viewer Hit Map Pane toolbar

BLAST Viewer Alignment Pane Toolbar


















Button	Description
	options for specifying technical settings

Table 4. 49 BLAST Viewer Alignment Pane toolbar

Database Explorer Toolbars

Database Explorer Main Toolbar

Button	Description
	back to previous document
	go to next document
	create a new subbase
	dismiss a subbase
	search the database
	creates a local or shared database exchange
	copy data to the clipboard
	paste data on a clipboard
	creates new object in the database objects pane
	edits object selected in the database objects pane
	renames the selected object
	deletes objects
	displays the properties of the selected object(s)
	view mode displays large icons, small icons, list and details

Button	Description
	copy an item to a file or the clipboard
	display online help

*Table 4. 50 Database Explorer Main toolbar***Xpression NTI: Expression Database Explorer Toolbars****Expression Database Explorer Main Toolbar**















Button	Description
	go to Expression Viewer
	back to previous subset
	go to next subset
	create a new subset
	dismiss a subset
	search the database
	creates new object in the database objects pane
	edits object selected in the database objects pane
	renames the selected object
	deletes objects
	displays the properties of the selected object(s)
	view mode displays large icons, small icons, list and details
	copy an item to a file or the clipboard
	display online help

Table 4. 51 Expression Database Explorer Main toolbar

Xpression NTI: Expression Viewer Toolbars

Expression Viewer Main Toolbar






















Button	Description
	go to Expression Database Explorer
	open a file
	save the current experiment to a file or to the database
	cut selection to the clipboard
	copy selection to the clipboard
	paste from the clipboard
	begin an Expression Database search session
	undo the last operation
	redo the last operation
	print preview the active pane
	print the active pane
	copy an item to a file or the clipboard
	display online help

Table 4. 52 Expression Viewer Main toolbar

Expression Viewer View Toolbar

Button	Description
	zoom in on the active pane
	zoom out the active pane
	fit image to pane window
	show/hide ruler toggle
	show/hide grid lines toggle
	tag the selection/add to a group

Button	Description
	untag the selection/remove from a group
	shows Distribution Map bin properties

*Table 4. 53 Expression Viewer View toolbar***Expression Viewer Tools Toolbar**






Button	Description
	data clustering options
	data sorting options
	data filtering options
	plot analysis options
	data processing options

Table 4. 54 Expression Viewer Tools toolbar

Chapter 5 Tutorial: Molecule Display Windows

Introduction

This tutorial chapter introduces you to the Vector NTI interface and Display windows, Vector NTI Suite's primary means for displaying and manipulating DNA/RNA/ protein molecules, gels, and other search and analysis features.

At the end of this tutorial session, you will be able to:

- Create and become familiar with Molecule Display windows for DNA and proteins
- Navigate the Vector NTI interface
- Manipulate molecule graphics, sequences, and Text Panes
- Review molecule data
- Select and examine molecule fragments
- Link molecule features for selective display in all panes
- Display amino acid translations of the nucleotide sequence and open corresponding proteins

Follow the steps of the tutorial in the order shown. Figures show what your screen should look like at various points along the way.

1. Launch Vector NTI

To launch Vector NTI, double-click its icon in the program group or folder where installed Vector NTI.

The first time you enter Vector NTI after installation, the database is empty. Vector NTI asks permission to import an initial set of database objects from Vector NTI archives. Press the **OK** button in the confirmation dialog boxes for the creation of all objects.

The DNA molecules, proteins, enzymes, oligos, and gel markers are imported to form the initial Vector NTI database.

2. Inspect the Vector NTI Workspace and Database Explorer Windows

Vector NTI first appears on the screen with an empty workspace, superimposed by the Database Explorer window.

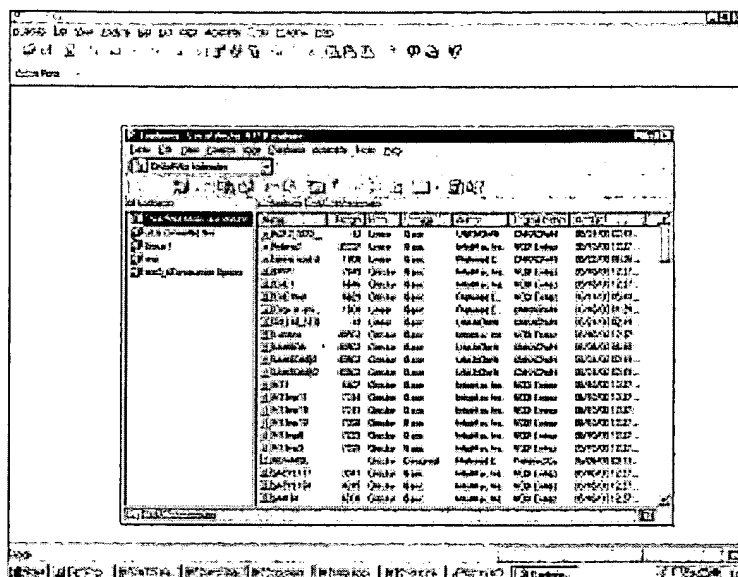




Fig. 5.1 Database Explorer window


The Database Explorer window (Fig. 5.1) shows the contents of a DNA/RNA molecules or Proteins database table. The Vector NTI database is a collection of molecules and laboratory data, organized for easy retrieval and management in Vector NTI's Database Explorer.

The Explorer window can be closed without exiting Vector NTI. You can activate or reopen the Database Explorer window at any time using the **Local Database** button () on the main toolbar of the Vector NTI workspace.

You will work extensively with the Database Explorer tutorial, chapter 8, but for now close the Explorer by clicking on the **Close** button () at the upper right of the title bar.

3. Create and Inspect a Molecule Display Window for pBR322

A Molecule Display window displays text information including a description of a molecule, features and a graphical map of the molecule and its sequence. Before proceeding any further, however, let's open a molecule in the so that you can review the features as they are discussed.

On the Molecule Display main (upper) toolbar, press the **Open** button (). In the Open dialog box, select the DNA/RNA Molecules tab. *The DNA/RNA (MAIN) database is selected in the drop-down menu.* On the molecule list, scroll to pBR322 and double click on it opening its Molecule Display window.

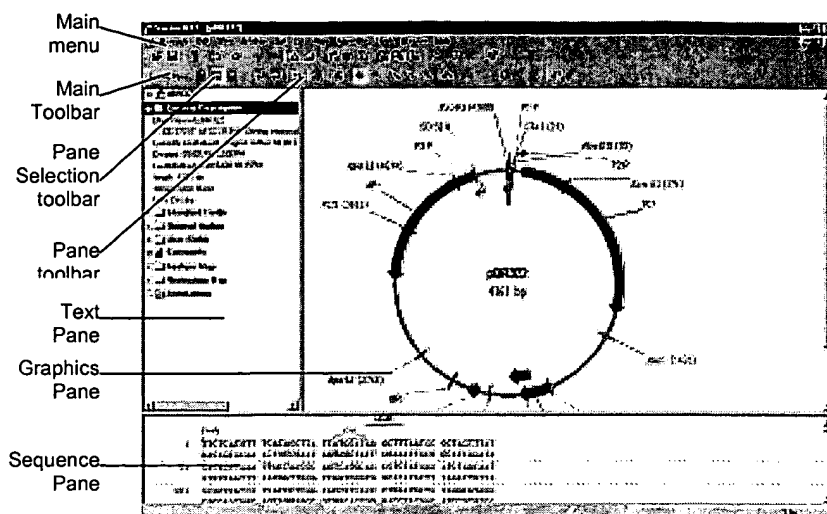



Fig. 5. 2 Molecule Display window for pBR322




A Molecule Display window title bar displays the name of the open object. In the display window that opens for pBR322 (Fig. 5.2), click the maximize box () in the upper right corner on the title bar. The window expands to fill the entire Vector NTI workspace.

A Molecule Display window is divided into three panes: a Text Pane (upper left) containing folders with descriptions of a molecule and analysis results, a Graphics Pane (upper right) displaying a graphical depiction of the molecule map including labeled features and restriction sites, and a Sequence Pane (lower) displaying the molecule's nucleotide or amino acid sequence.

All Vector NTI Suite windows have a Main Menu and toolbars where many different options and operations can be initiated. While many toolbar buttons are consistent in all display windows, many toolbar buttons are context-sensitive. All Vector NTI Suite toolbar buttons are summarized in Chapter 4 of this manual. As you are introduced to various operations in the tutorial chapters, however, the toolbar buttons you will use are displayed appropriately.




4. Arrange the Display Window Conveniently

To modify the relative sizes of the display panes, you can move the vertical and horizontal split bars. Move the cursor arrow to the split bar where it changes to a two-ended arrow. CLICK + DRAG with the left mouse button to move the split bar. Additionally, each pane has its own scroll bars when the pane content exceeds its display area.

To activate the different panes of the display window, click the Text Pane () , Graphics Pane () or Sequence Pane () buttons on the Window Toolbar. (You can also activate each pane by clicking it.) *Note how the Windows Toolbar changes, giving you different tools for each pane.*

Switch to the Graphics Pane. Move the horizontal and vertical split bars to enlarge the Graphics Pane viewing area.

Click on each of the following buttons to resize the graphical map:

- The Zoom In button () enlarges the image
- The Zoom Out button () reduces the image
- The Fit to Window button () fits the image to the size of the window.

Now reduce or increase the map size in increments by holding down the SHIFT key and clicking the Zoom buttons. Using the scroll button on your mouse while holding down the shift key has the same effect as clicking the Zoom buttons.

5. Select a Fragment or a Feature in the Graphics Pane

Now let's study Vector NTI's selection techniques.

Make sure the Graphics Pane is active. Choose **Edit > Set Selection** on the menu bar, opening the Set Selection dialog box. Enter the range 100 bp – 1000 bp and click **OK**.

A selection wireframe highlights the fragment on the molecule map (Fig. 5.3), with a caret (short darkened line perpendicular to the wire frame) marking one end of the wireframe.

The selection position is displayed in the status bar at the bottom of your screen. The caret position is also indicated on the status bar. The selection is simultaneously highlighted in the Sequence Pane.

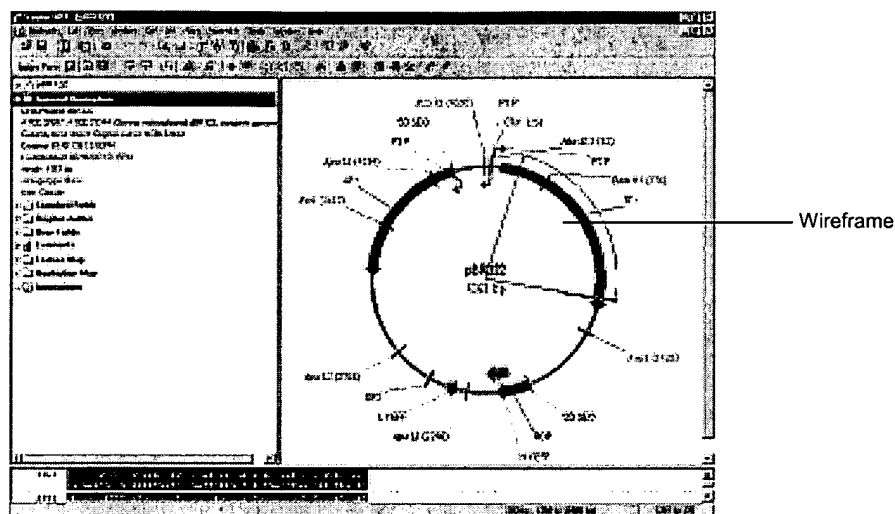



Fig. 5.3 A selection wireframe highlights the fragment on the molecule map

Modifying the Selection

Click on the status bar with the mouse, opening the Set Selection dialog box again. Change the setting to 200 bp – 2000 bp.

You can reposition the 5' or 3' ends with the mouse by “dragging and dropping”. Move the cursor to the 5' end, identified when the cursor changes shape to a crosshair with the 5' mark ().

Click and drag the 5' caret to a new position near the start of the molecule (“12:00 o'clock”).

Hold the Shift key down and press the right or left keyboard arrows. *This moves the cursor 1 nucleotide (NT) at a time. Follow this in the Status Bar.* Move the 5' end to “1 bp”.

Press the END key to move the caret to the 3' end (or click the cursor on the 3' end). Press and hold the SHIFT key while moving the left or right arrows to modify the selection. Move the 3' end to 1250 bp.


Hold down the SHIFT + CTRL keys and press the right or left arrow several times. *Note that the caret now moves 10 NT at a time.* Release the CTRL key, hold down SHIFT and press the left arrow. *The caret is moving 1 NT at a time again.* If you hold down the arrow keys while holding the SHIFT key, the caret moves rapidly through the sequence. Release the SHIFT key and press HOME. *The caret returns to the 5' end.*

To make a new selection, click anywhere in the blank area inside the molecule map, selecting the entire map. Click on the caret and drag the wireframe clockwise. Release the mouse.

Tip: If you wish to deselect everything and are having problems doing so in the Graphics Pane, click anywhere in the Sequence Pane.

Selecting Features

Locate the tetracycline resistance gene, TC®, on the graphics map. Pause the cursor over the functional segment or label for a moment. *A pop-up message with details about the functional signals appears under the cursor.*

To simplify selection of a functional signal on a graphical map, the paused cursor changes to a hand (). Click once over the TC®, selecting the gene. *A wireframe selecting the gene appears and the selected signal changes color. The gene sequence is concurrently selected in the Sequence Pane.*

Selection Techniques Summary for the Graphics Pane

When making or altering fragment selections, there are several general points to remember:

- Click and drag the mouse to select a region
- Click on a feature to select it
- Press SHIFT + right or left arrow to adjust an existing selection one nucleotide at a time.
- Press CTRL + SHIFT + right or left arrows to move the caret 10 nucleotides at a time.

Note: A selected fragment is simultaneously highlighted on the Sequence Pane. Vector NTI always displays selection in both panes no matter where you make the selection.


6. Examine pBR322's Nucleotide Sequence

Now let's work with pBR322's nucleotide sequence.

Drag the horizontal split bar up for better viewing of the nucleotide sequence. The nucleotides are displayed in 10 NT blocks. Restriction sites appear by name above their recognition sites. Motifs and ORFs also can be displayed by turning those options on the Molecule Display Setup to be discussed later in this chapter.

Click anywhere in the Sequence Pane to activate it. Any prior selection disappears and the blinking caret is placed at the position where you placed the cursor.

When the DNA/RNA Display Window opens, the displayed sequence is double stranded.

Click on the **Double/Single Strand** button () to change to a single stranded sequence display (Fig. 5.4).

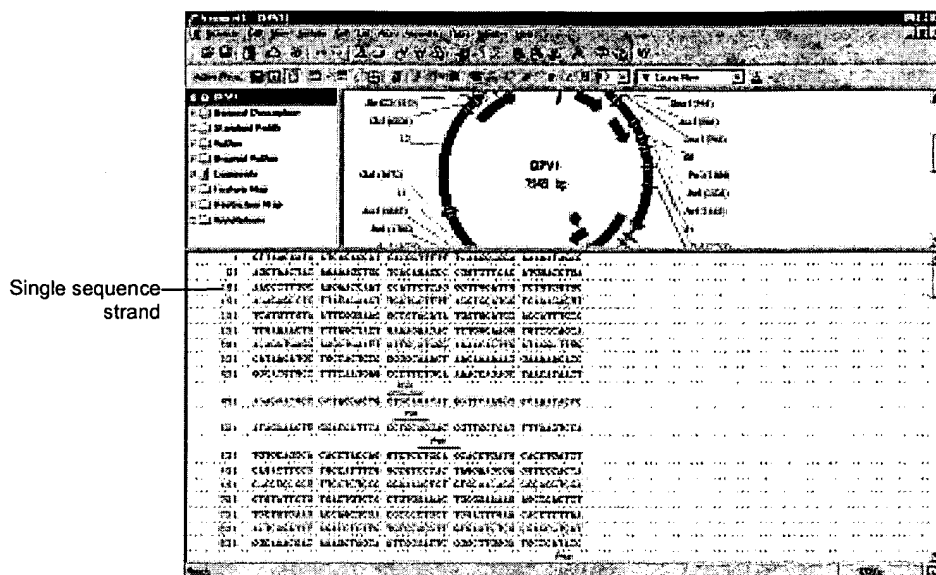




Fig. 5. 4 Single-stranded sequence display

Scroll to the top of the sequence, using the scroll bar to the right of the sequence.

Select the first few lines of the sequence by clicking and dragging the mouse left to right along the line and down through the sequence. The nucleotides are highlighted as you drag and their coordinates are displayed in the selection box on the status bar. *The selection also shows within a wireframe in the Graphics Pane.* Release the mouse.

To translate the selected sequence into amino acids, press the **Translate Direct** () and **Translate Complementary** () buttons. The corresponding amino acids appear above (direct strand) and below (complementary strand) the highlighted sequence (Fig. 5.5).

Numerals to the left of the sequence row indicate the phase of the translations. For the Direct strand translation, phases are determined by the position of the first selected nucleotide relative to the beginning of the molecule. They are designated +1, +2, or +3. Complementary strand translation phases are determined by the position of the last selected nucleotide relative to the end of the molecule. They are designated -1, -2, or -3.

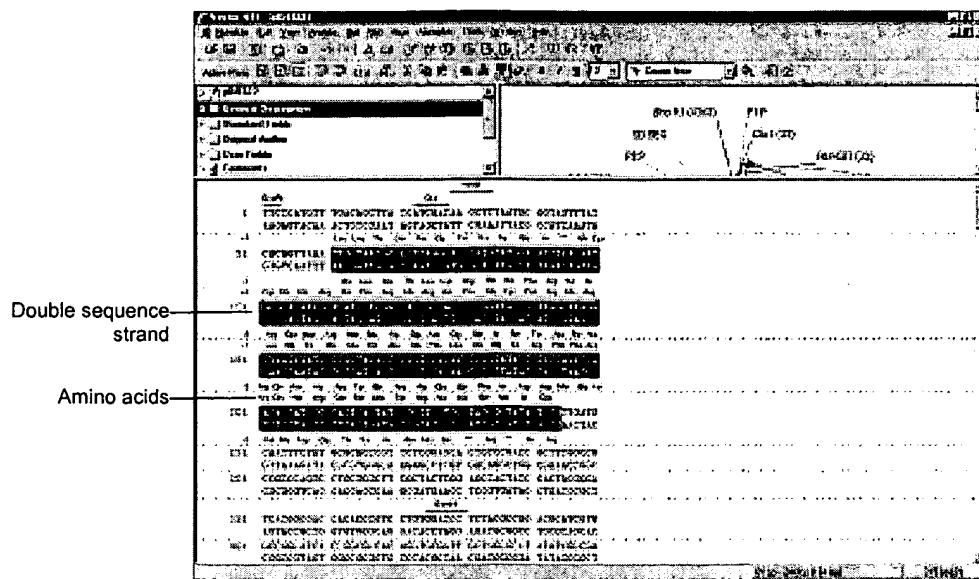



Fig. 5. 5 Selected double sequence strands translated into amino acids

To change from the 3-letter amino acid code to a 1-letter code, choose the **Display Setup** button  and select **Display Setup** on the dropdown menu.

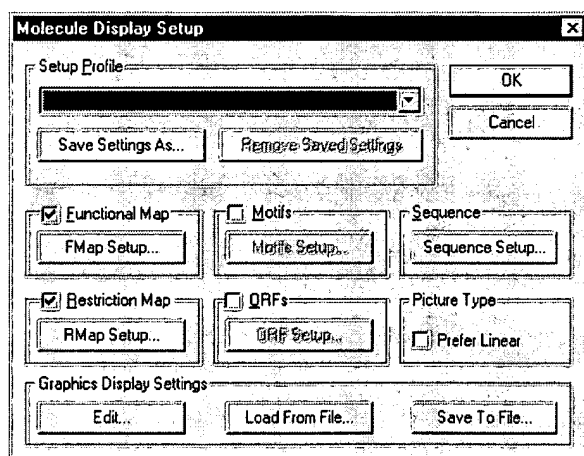


Fig. 5. 6 Molecule Display Setup dialog box


In the Molecule Display Setup dialog box (Fig. 5.6), choose the **Sequence Set-up** button and then the 1-letter code radio button in the Sequence Setup dialog box. Click **OK** in both

dialog boxes. *Inspect your selection.* This dialog box is also used to modify or specify the display of other features such as restriction sites, motifs and ORFs.


Click on the **Erase Translations** button () on the toolbar to erase the translations.


7. Work with pBR322's Text Description

Click in the Text Pane or click the **Text Pane** button to activate the Text Pane. Drag the vertical split bar to the right to enlarge the Text Pane.


The Text Pane is made up of folders that can be opened or closed by double clicking or clicking on the () to the left of the folder name. Open and review the contents of each of the folders. Note particularly the following:

General Description Folder: This folder contains the molecule description. Close the folder by clicking on it.

Feature Map Folder: Select this folder and press the **Expand Branch** button () opening all of the Feature map subfolders. Move through the subfolders until you come to the TC® in a subfolder of the CDS (Coding DNA Sequence) folder. *Note the coordinates shown.*


Select the TC® folder and press the **Find** button () *This selects the gene in the Sequence and Graphics Panes.* Close the folders with a double click.

The Find button can also be used to locate ORFs, motifs and other objects. Select the appropriate folder in the Text Pane and press the **Find** button again. *The keyboard equivalent is CTRL + F.*

Restriction Map Folder: Click on this folder to open it. Click on the **Expand Branch** button () in the Window Toolbar, opening all restriction map subfolders.

Each subfolder contains a restriction site, listed alphabetically. Restriction sites which are present at least once on pBR322 have active (colored) folders showing all of the site's positions on the molecule and its recognition sequence. To the right of each enzyme's name, its recognition sequence and cleavage points are displayed. Inactive folders (grayed out) are for restriction endonucleases with no sites on the molecule.

The set of restriction enzymes listed in the Text Pane and searched for on all DNA molecules when you open them is defined in the Molecule Display Setup dialog box. To

examine the list of selected enzymes, press the **Display Setup** button () on the Window Toolbar and choose **Display Setup** on the dropdown menu. Press the **Rmap Setup** button, opening the Restriction Map Setup dialog box (Fig. 5.7).

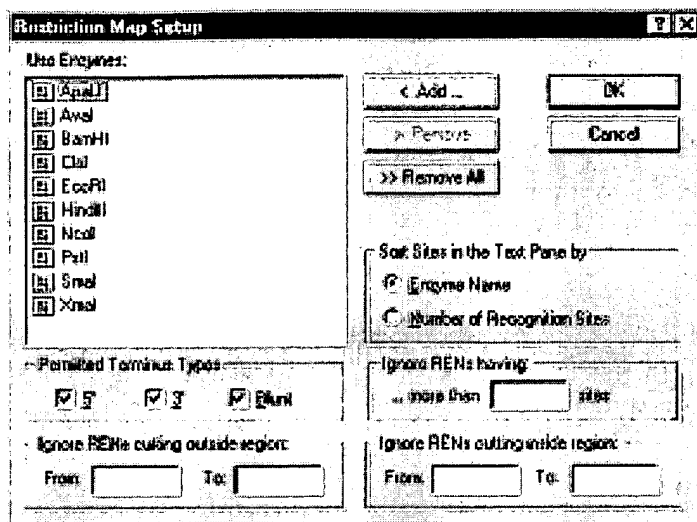



Fig. 5. 7 Restriction Map Setup dialog box


In this dialog box, you can add (or remove) any enzymes you wish to be searched for on all open DNA molecules. Do not change the default settings. Press **Cancel** in both dialog boxes to return to the Display window.

Locate the *ApaLI* folder in the Text Pane. The folder is open, showing a list of all *ApaLI* sites on the pBR322 molecule. Click on the first site line to select it and press the **Find**

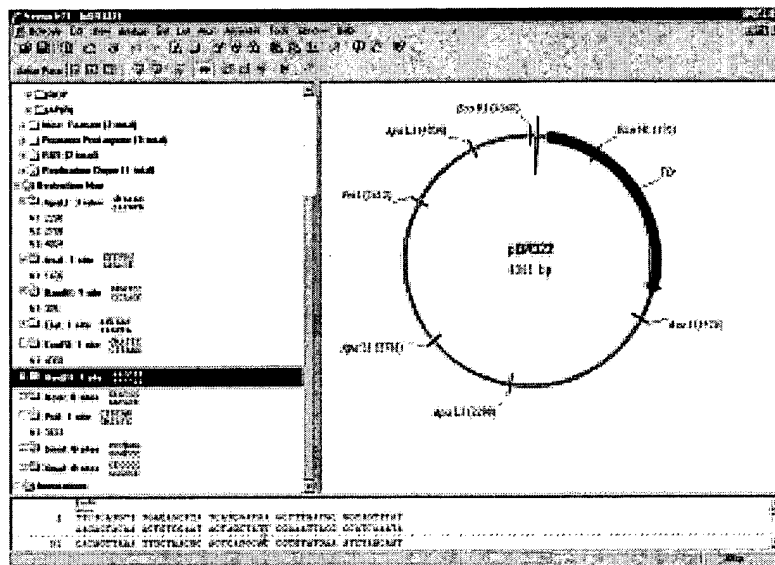
button () on the Window Toolbar. *The ApaLI site is selected on the Graphics Map and in the Sequence Pane.* Move to those panes to verify this, then return to the Text Pane. Close the Restriction Map Folder with a double-click.

8. Link pBR322's Text Pane to the Graphics and Sequence Panes

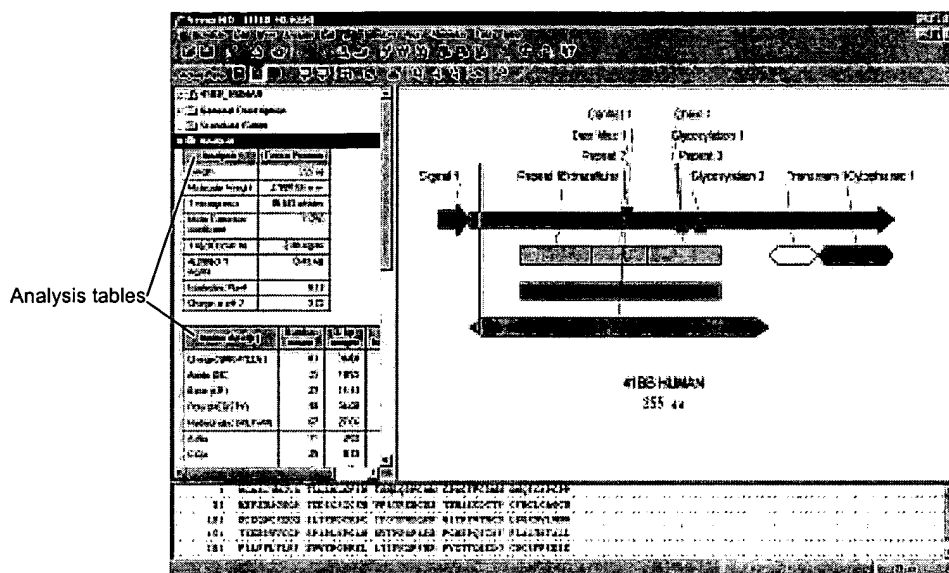
In Vector NTI's Display Windows, you can link panes so that the graphics and Sequence Panes display only those objects whose folders are open in the Text Pane.

Press the **Link Panes** button () on the Window Toolbar. *Most of the information disappears from the graphical map, leaving only the molecule name and length.* In the Text Pane, open the Feature map folder and only the subfolders for the TC® gene (in the CDS subfolder) and the Restriction Map folder with only the following enzyme folders open: *ApaLI*, *AvaI*, *BamHI*, *EcoRI* and *PstI*. Because only open folders are now linked with the Graphics Pane, only those features are illustrated in the Graphics Pane. (Make sure the folders are closed for any features you do not want displayed.) *Note that the sites and functional signals appear on the graphic map as you open them in the Text Pane. The sequence fragments are also highlighted at the same time.*


Toolbar () to rearrange the restriction site labels for optimum display (Fig. 5.8).



Double-click on the 41BB_HUMAN molecule in the Database Protein Molecules list in the right pane. A Molecule Display window opens for 41BB_HUMAN (Fig. 5.9) containing its text description, analysis results, graphical Feature map, and sequence:



Double-click on the Analysis folder in the Text Pane, opening two tables showing the results of the automatic protein analysis.

Press the **Camera** button () or choose **Edit > Camera** on the menu bar. In the Camera dialog box, choose the range **Selection** and destination of your data **Clipboard**.

Press the **Copy** button, switch to a word processor program and paste the result into a new or existing document. *The results are displayed in a standard tabular form as shown here, (in a truncated version):*

Analysis	Entir Protein
Length	255 aa
Molecular Weight	27897.66 m.w.
1 microgram =	35.845 pMoles
Molar Extinction coefficient	11250
1 A[280] corr. to	2.48 mg/ml
A[280] of 1 mg/ml	0.40 AU
Isoelectric Point	8.13
Charge at pH 7	3.72

Table 5. 1 Results of an automatic protein analysis in a word processor program

Amino Acid(s)	Number count	% by weight	% by frequency
Charged (RKHYCDE)	83	33.00	29.00
Acidic (DE)	25	10.00	9.00
Basic (KR)	29	13.00	11.00
Polar (NCQSTY)	90	32.00	31.00
Hydrophobic (AILFWV)	67	24.00	24.00
A Ala	11	3.02	4.31
C Cys	25	9.33	9.80
D Asp	11	4.51	4.31
E Glu	14	6.34	5.49
F Phe	16	8.14	6.27

Table 5. 2 Results of an automatic protein analysis in a word processor program


11. Create a Display Window for 1B14_HUMAN

Return to the Database Explorer window, the Protein Molecules table and select the Protein Molecules (MAIN) subbase.

Double-click the 1B14_HUMAN protein in the molecules list, opening it in the Molecule Display window. Arrange the panes conveniently.

Note that with a large Feature map, the Graphics Pane appears crowded. To make it more convenient to work with, use the "link mode" described above in step 8 to display fewer features. Alternatively, Vector NTI allows you to limit the view by choosing a fragment of the molecule for closer inspection (Fig. 5.10).

Select the region 231 aa –276 aa using the techniques described for DNA molecules.

Click on the **View Molecule Fragment** button  and choose **View Selection (231 aa – 276 aa)** from the popup menu. All three views are rearranged to display only the selected fragment.

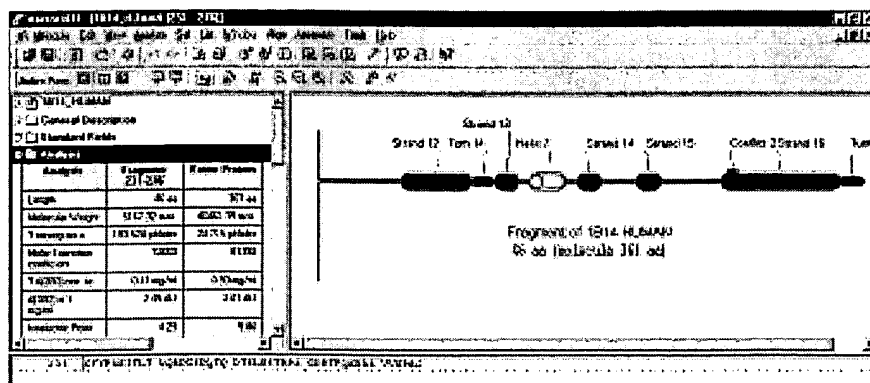


Fig. 5. 10 Viewing a molecule fragment

Double-click on the Analysis folder in the Text Pane. Note that the analysis tables now show values for the selected fragment as well as for the entire protein.

Most of the other techniques for manipulating Protein Molecule Display windows are exactly the same as for DNA/RNA Display windows.

12. Close the Display Windows and Exit Vector NTI

To complete your first session with Vector NTI, select **Molecule > Close** on the menu bar, closing the display windows.

To exit Vector NTI, select **File > Exit**.

Chapter 6 Tutorial: Molecule Editing

Introduction

This chapter introduces you to operations of Molecule Display Windows that allow you to edit a molecule's Feature map and sequence. Although in this chapter you will work only with DNA molecules, all operations are applicable to protein molecules as well.

At the end of this tutorial session, you will be able to:

- Create a Molecule Display window for a molecule
- Edit the general data, Feature map, and sequence for a DNA molecule
- Rename, describe and save a molecule in the database
- Alter starting coordinates for a DNA molecule

Follow the steps in the order shown. Figures show what your screen should look like at various points along the way.

1. Launch Vector NTI

Launch Vector NTI by double-clicking its icon in the program group or folder in which you installed Vector NTI.

2. Open a Display Window for pBR322

Activate the Database Explorer window and switch to the DNA/RNA molecules table.

Select the DNA/RNA Molecules (MAIN) subbase and double-click on the pBR322 molecule.

A display window opens in the workspace, containing pBR322's text description, graphical features, and restriction maps, and sequence. Maximize the Display window and arrange its panes conveniently.

3. Edit pBR322's General Data

At the top of the Text Pane, double-click the pBR322 name. This opens the Edit pBR322 dialog box (Fig. 6.1):

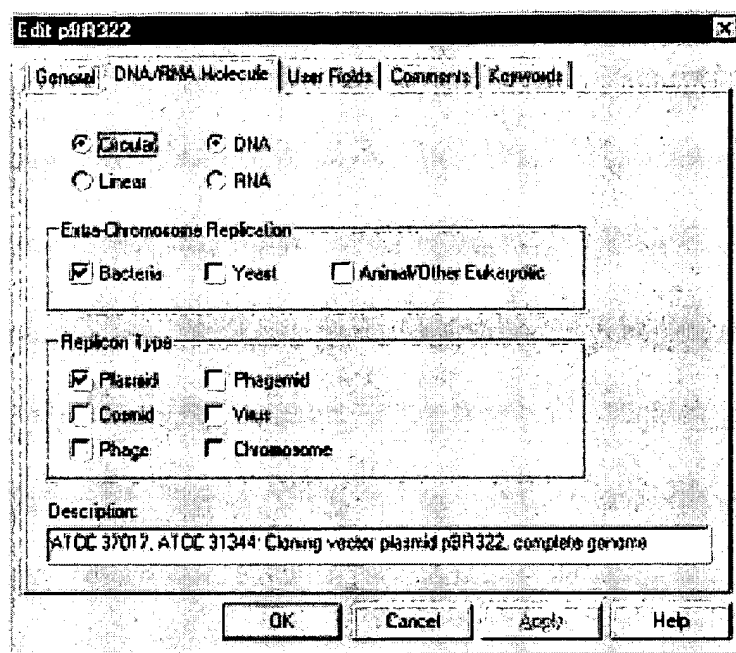



Fig. 6. 1 Edit pBR322 dialog box (DNA/RNA Molecule tab shown)

First, let's add a keyword to the list of pBR322's keywords. Choose the Keywords tab and enter **My own plasmid** in the keyword drop-down menu. Press the **Add** button. Now the keyword list contains two keywords: "ATCC" and "My own plasmid". Switch to the DNA/RNA Molecule tab, and change the description of the molecule to "My pBR322." Press the **OK** button.

Vector NTI updates the General Description folder in the Text Pane to show the new properties. An asterisk now appears on the title bar, indicating that the molecule in a window is a modified version of the pBR322 molecule stored in the Vector NTI database.

To save the modified pBR322 into the database under a new name, select **Molecule > Save As** from the menu bar and enter **My pBR322** to the DNA/RNA text box. Press **OK**.

The modified molecule is stored under the new name and updated in the display window. The title bar and both the text and Graphics Pane show the new name "My pBR322". The title bar is no longer marked as modified (asterisk) because the displayed molecule is exactly the same as the database molecule "My pBR322".

Open a Display window for pBR322 to make sure that the original molecule was not modified. To do so, click the **Open** button () on the Main Toolbar and select pBR322

on the Database DNAs/RNAs page. To continue, close all extra Display windows except “My pBR322”.

4. Edit My pBR322's Sequence

Activate the Sequence Pane and select the fragment 21 bp–40 bp (Fig. 6.2) using either the drag and select method or the Set Selection dialog box described in the previous chapter. Note that the selected sequence contains *Cla*I and *Hind*III sites.

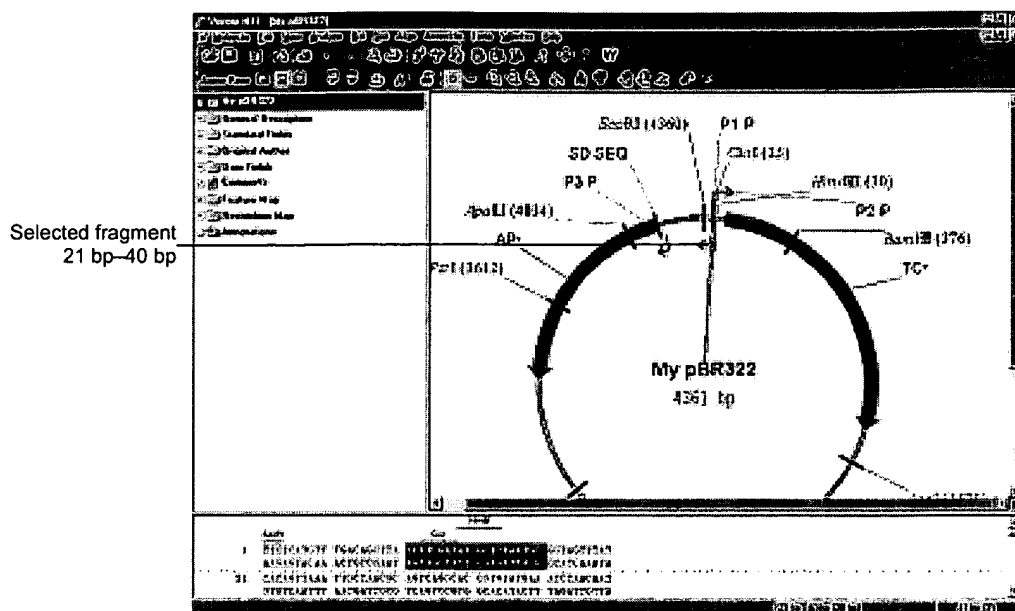


Fig. 6. 2 Display window for pBR322 with selected fragment 21 bp–40 bp

Choose **Edit > New > Replace Sequence 21 bp–40 bp** from the menu bar. In the Replace Sequence dialog box (Fig. 6.3), use arrow keys to move the caret to the position “before 26 bp” (the caret position is displayed at the bottom of the dialog box). Press the **Backspace** key twice to delete C and T to the left. Type A twice so that the resulting sequence is TCAAAGATAAGCTTTAATGC. The status line on the bottom of the dialog box displays “inserted 2, deleted 2” message.

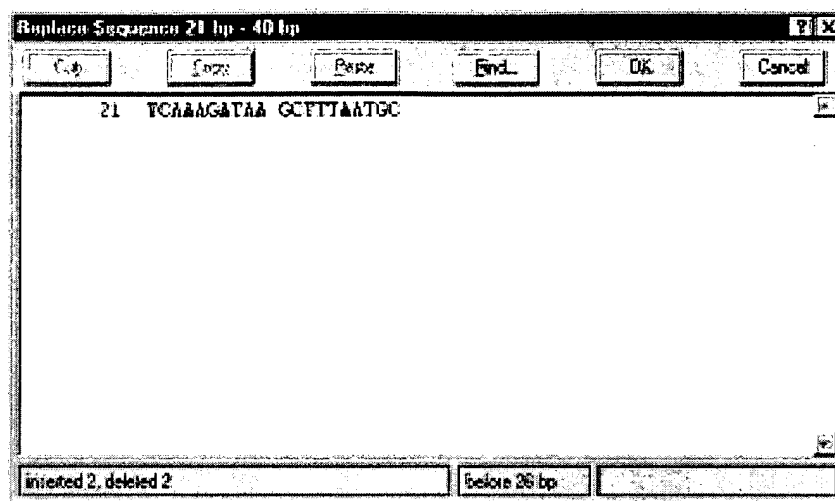


Fig. 6.3 Replace Sequence dialog box

Press the **OK** button to finish editing the fragment. In the updated Display window (Fig. 6.4), the ClaI site on the modified fragment has disappeared.

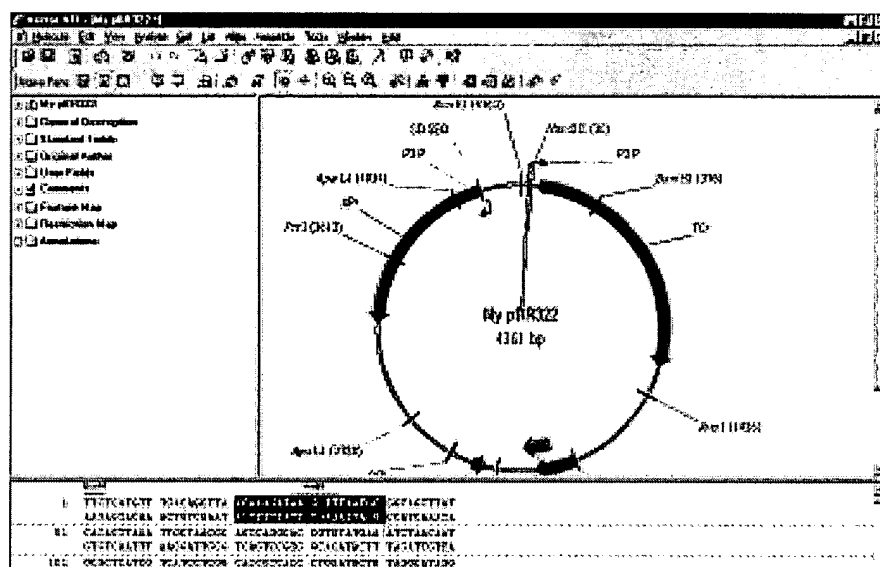


Fig. 6.4 Display window with updated sequence

5. Revert to Database Version of My pBR322

The “My pBR322” molecule in the display window is now flagged (with an asterisk) as modified because it differs from the version of “My pBR322” currently saved in the database.

To return to the database version, choose **Molecule > Revert To Saved In Database** from menu bar. Press **OK** in the confirmation dialog box.

The molecule in the display window returns to its database state and the window is no longer marked as modified (no asterisk). Notice that *Cla*I site returned to its place in the original sequence.

6. Insert a New Sequence Fragment

In general, editing a molecule sequence requires the adjustment of its Feature map. In this step we will insert a new sequence fragment into our molecule and look at the resulting Feature map.

Before you start, notice that the current AP(R) position is 3293 bp–4156 bp; the TC(R) position is 86 bp–1276 bp. To see the positions of the features, pause the mouse cursor over the feature or its label on the graphics map. A popup label appears, displaying the name and position of the feature under the cursor.

Place the caret at 200 bp using the mouse, arrow keys, or **Edit > Set Caret Position**. Choose **Edit > New > Insert Sequence at 200 bp** from the menu bar, opening the Insert Sequence dialog box. Type in ten Ts (the number of nucleotides you entered is shown at the bottom of the dialog box). Press **OK** to insert the sequence.

Vector NTI figures out that the modification you made requires an adjustment in the Feature map. The dialog box appears with the message “CDS TC(R) is affected by sequence editing.” Press the **Keep** button to leave the feature in a Feature map. Note that the inserted nucleotides are highlighted in the sequence (Fig. 6.5).

Place the mouse cursor over AP(R) again to determine its position. Note that AP(R) has been moved 10 nucleotides clockwise and is now positioned at 3303 bp–4166 bp.

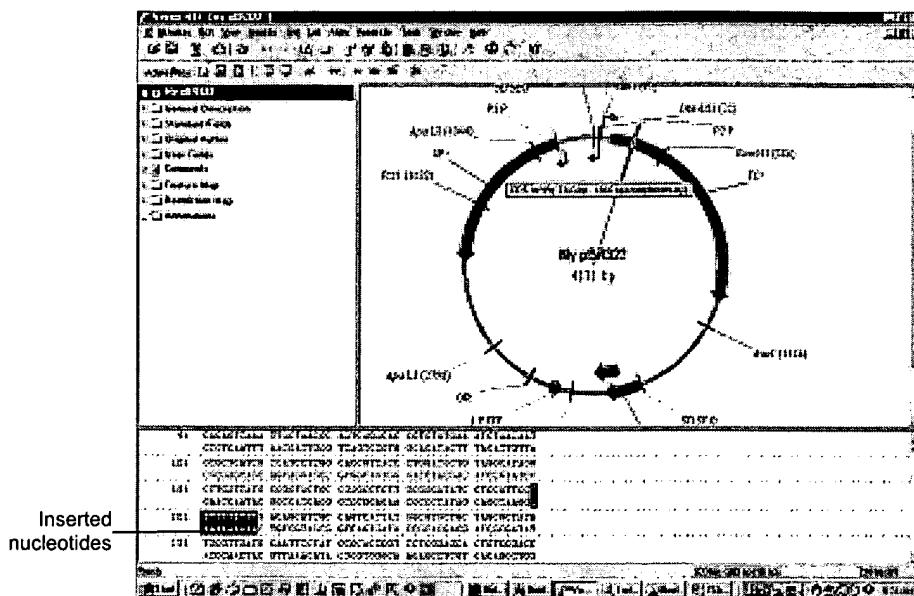


Fig. 6. 5 Inserted nucleotides are highlighted in the sequence

Vector NTI automatically moves all features located after the insertion but it does not move any features before the insertion. If the insertion point is inside a feature and you decided to keep the feature, Vector NTI automatically moves only the 3' end of the feature. Observe that the 3' end position of TC(R) was changed to 1286 bp.

7. Edit the TC(R) Signal

Double-click on the TC(R) signal or its name in the Graphics Pane. (The mouse cursor changes to a hand over features and their names). Alternatively, click on the signal once and choose **Edit > Feature Properties** from the menu bar or choose **Feature Properties** from TC(R)'s shortcut menu. (To display a shortcut menu for any object, right click on the object. The shortcut menu contains commands appropriate for the item to which you are pointing.)

In the Molecule Feature dialog box that opens, you can change the name, type or description of the feature as well as its position on the molecule. Change the name of the signal to "Old TC(R)" and its description to "10 bp fragment inserted". Press the **OK** button. Vector NTI updates the Display window to show the new name of the signal (Fig. 6.6).

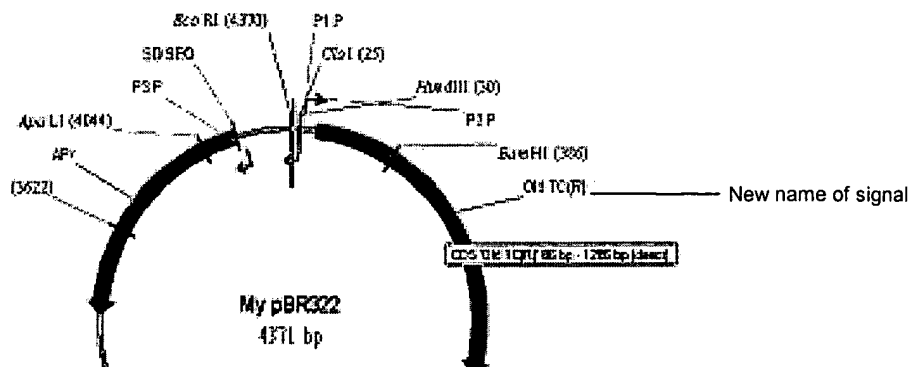



Fig. 6. 6 Display window showing new name of the signal

8. Delete the P2_P Signal and Add a New Signal

Vector NTI allows you to easily add and delete features in a Feature map. To delete the P2_P promoter from the Feature map, select it with a click on the label in the Graphics Pane and choose **Edit > Delete Feature From FMap** from the menu bar. Alternatively, select P2_P and choose **Delete Feature From FMap** from the shortcut menu. Press the **OK** button in the confirmation dialog. P2_P is deleted from the Feature map and disappears from the Display window.

Let's add a new feature to the Feature map of My pBR322. First, select region 3000bp–3500

bp and press the **Add Feature** button () on the Window toolbar. Alternatively, you can choose **Edit > New > Add Feature to FMap**. In the Molecule Feature dialog box, Vector NTI puts the currently selected region into the From and To fields. By default, the "Misc. Feature" type is assigned to new DNA/RNA features in the Feature Type panel, but you may change the type to any other type. Name the new feature **New Feature** and press the **OK** button. The new feature appears in the text and Graphics Panes of the Display window (Fig. 6.7).

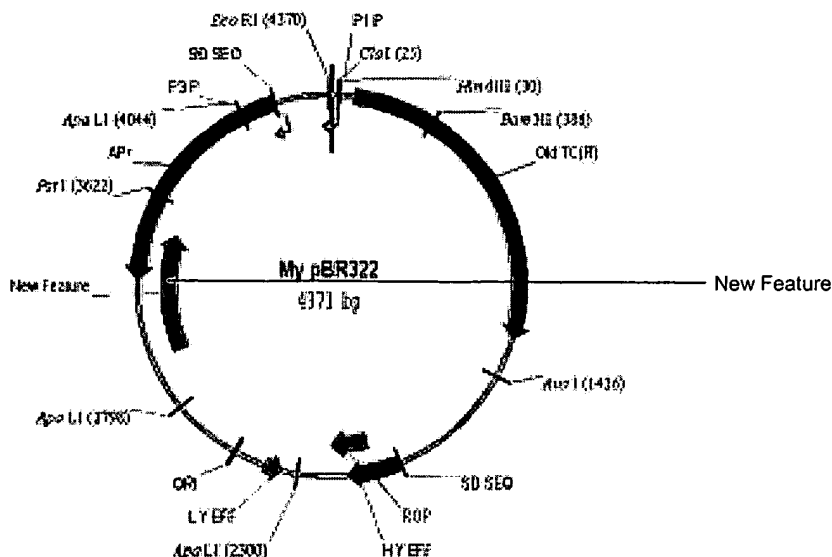


Fig. 6. 7 New feature appears in the Graphics Pane

To save the modified My pBR322 into the database, choose **Molecule > Save As** from the menu bar. Press the **OK** button, then **OK** again to overwrite the version of My pBR322 currently stored in the database.

9. Change the Starting Coordinate of My pBR322

The Molecule menu options control the operations that can be performed on a molecule as a whole. Let's change the starting coordinate of My pBR322 so that all features *after* the inserted fragment have the same coordinates they have in the original pBR322 molecule. Choose **Molecule > Operations > Advanced (DNA/RNA) > Change Starting Coordinate** from the menu bar. In the dialog box that opens, enter a new starting coordinate. Since the length of the inserted fragment was 10 bp, the new starting coordinate should be 1 bp + 10 bp = 11 bp. Enter 11 into the New Start field and press the **OK** button. Press **OK** in the confirmation dialog. Vector NTI recalculates the Feature map and updates the Display window. Verify that the new coordinates of AP(R) feature are the same as in the pBR322 (Fig. 6.8): 3293 bp–4156 bp and "Old TC(R)" is now at 76bp–1276bp.

Notice that the Display window is not marked as modified. For all commands from the Molecule menu, Vector NTI always operates on the molecule in the database and updates the Display window if required. If a molecule in a Display window was modified but not saved at the time you choose a command, Vector NTI asks you to save the modified molecule into the database and then repeat the command.

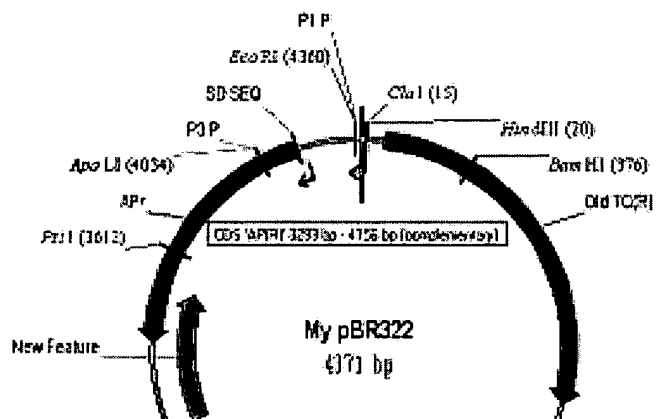


Fig. 6. 8 New coordinates of AP(R) feature

10. Close the Display Window and Exit Vector NTI

You have finished your session with Vector NTI. Close the Molecule Display window using the Close command option in the Molecule menu. Quit Vector NTI with the File menu's Exit command.

Chapter 10 Tutorial: Molecule Construction

Introduction

This chapter introduces you to creation of a new DNA molecule by *Construction*. In Vector NTI terminology, Construction means creating a DNA molecule from fragments that are completely defined and made compatible by the user.

At the end of this tutorial session, you will be able to:

- Use Fragment Wizard to define the needed fragments for the Goal Molecule Definition List
- Name and describe the new molecule in the Construct Molecule dialog box
- Verify that the fragments are correctly defined
- Direct Vector NTI to construct your new molecule
- Modify the fragment termini biochemically, when necessary, to allow completion of the Construction process

DNA molecules can be composed of: fragments of existing DNA molecules, linkers, adaptors, dummy fragments and so forth. Most of the fragments you will use to create new molecules are fragments of existing molecules, and most of the work involved in creating a new molecule is in defining them. Fragments like linkers and adaptors are much easier to describe.

The Fragment Wizard guides you through the process of describing a new molecule fragment. In addition to using the Fragment Wizard, you can define fragments using the Fragment Editors available from the Construct / Design Molecule dialog box. While they can describe fragments of all types, Fragment Editors are most convenient for defining linkers, adaptors, and so forth.

Molecules that you or Vector NTI create from fragments of existing molecules are called constructed molecules. Molecules that you import or describe by hand are called basic molecules because they enter the database as complete units rather than being built in Vector NTI from fragments.

For more information regarding other methods of adding new molecules to Vector NTI's database, such as design, importing molecules, assembling a molecule from a sequence text file, refer to Chapters 11 and 12.

Follow the steps in the order shown. Figures show what your screen should look like at various points along the way.

1. Launch Vector NTI

Launch Vector NTI using techniques you learned in previous tutorials.

2. Create Display Windows for pBR322 and pUC19

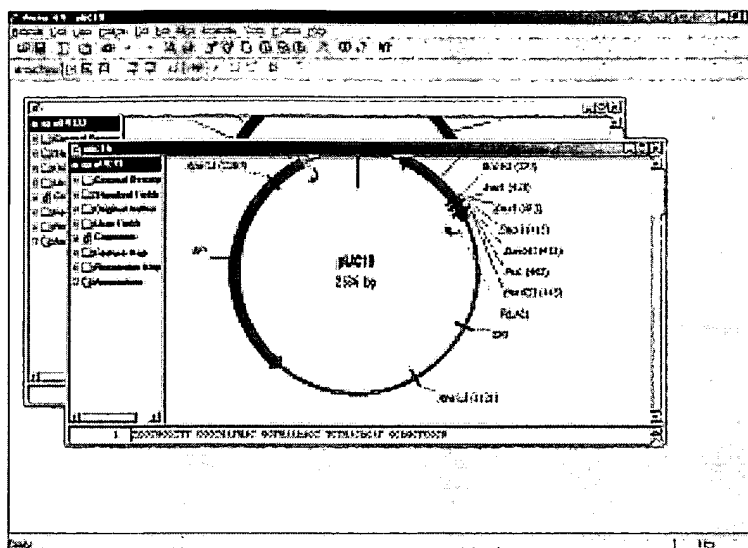


Fig. 10.1 Opening two Display Windows


Using the techniques you learned in previous tutorials, click the **Open** button and open pBR322. Then click the **Open** button again to open pUC19. Two display windows are now open, one for each plasmid (Fig. 10.1). You can move from one to the other by selecting the molecule in the drop-down Window menu on the Main Toolbar. Maximize each window.

3. Arrange the Display Windows

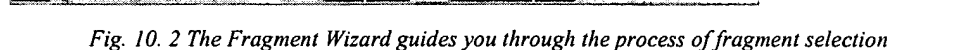
Expand the working space for the Graphics and Sequence Panes by reducing the Text Pane in each Display window. Adjust the graphical map for each plasmid so you can easily read the features and labels.

4. Define the First Fragment (from pUC19)

The first fragment of the goal molecule includes most of pUC19, with the 5' end of the fragment being the *Sma*I restriction site and the 3' end being the *Eco*RI restriction site. The second fragment is from pBR322, the 5' end being the *Eco*RI site and the 3' terminus being the *Ava*I restriction site.

Activate the Graphics Pane of the pUC 19 Display window. Click the **Add Fragment to Molecule Goal List** button () to open the Fragment Wizard (Fig. 10.2).

This dialog box guides you through the process of fragment selection, with several sequential screens describing the corresponding actions you should take. If you make a mistake you can return to the previous step using the **Back** button. You can drag the dialog

[illegible]

- 2) **Number of Chromosomes** 4n = 16 (1: 8%)



- **Second screen (Fig. 10.3):** To determine the 5' terminus of the new fragment, click on the *Sma*I restriction site label in the Graphics Pane. The Set to a Restriction Site option is automatically checked and the name and position of the restriction site is displayed in the Fragment Wizard. Click the Next button in the dialog box to proceed.

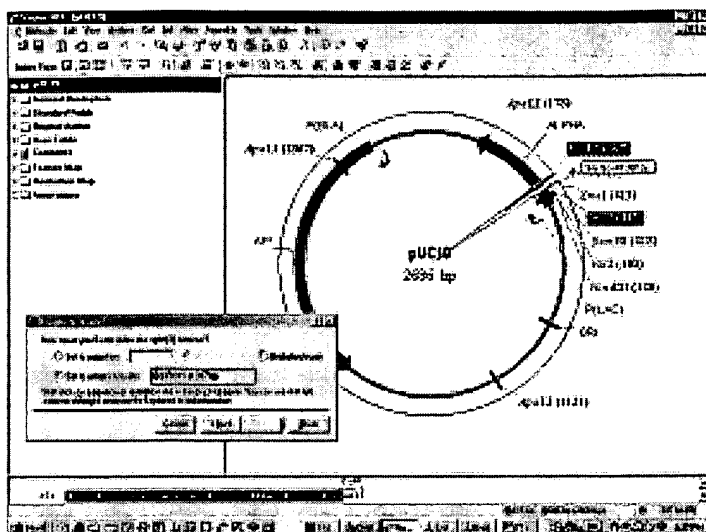


Fig. 10. 4 The third screen of the Fragment Wizard determines the 3' terminus

- **Third screen (Fig. 10.4):** To define the 3' terminus of a new fragment, hold the **SHIFT** key and click on the *Eco*RI restriction site label in the Graphics Pane. The Set to a Restriction Site option is automatically checked and the name and position of the 3' end are displayed.

Note: If you do not hold down the shift key when selecting the 3' end, Fragment Wizard reverts to choosing the 5' site.

Both labels in the Graphics Pane are now highlighted. The selected fragment is indicated by the wireframe in the Graphics Pane. Click the **Finish** button in this screen to complete the definition of the fragment.

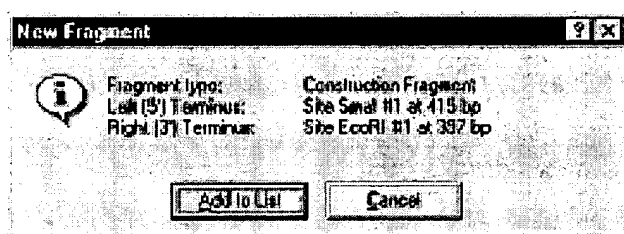



Fig. 10. 5 Adding the first fragment to the Molecule Goal list

Check the description of the fragment in the New Fragment message box: 5' *Sma*I, and 3' *Eco*RI. If there is an error, press the **Cancel** button and return back to the Fragment Wizard. Otherwise, press the **Add to List** button, adding the first fragment to the Molecule Goal list (Fig. 10.5).

5. Define the Second Fragment (from pBR322)

On the toolbar, select **Window > pBR322** which opens the corresponding Molecule Display window. Activate the Graphics Pane. Press the **Add Fragment to Molecule Goal List**

button () to display the Fragment Wizard dialog box again. Move the dialog box so that you can see the *Eco*RI and the *Ava*I restriction sites on the graphical map.

- **First screen:** Select the **Construction Fragment** option. Leave the Insert Inverted option unchecked, and click the **Next** button to proceed.
- **Second screen:** To determine the 5' end of the new fragment, click on the *Eco*RI restriction site label in the Graphics Pane. The Set to a Restriction Site option is automatically checked, and the name and position of the restriction site are displayed. Click the **Next** button to proceed.


Third screen: To define the 3' terminus of the new fragment, hold down the SHIFT key and click on the *Ava*I restriction site label in the Graphics Pane. The Set to a Restriction Site option is automatically checked and the name and position of the restriction site are displayed.

Click the **Finish** button to complete the definition of the fragment.

In the New Fragment message box, verify the fragment definition: 5' *Eco*RI and 3' *Ava*I. If the description of the fragment is correct, press the **Add to List** button. The second fragment is added to the Molecule Goal list.

6. Inspect the Goal List

To verify that the fragments in the Goal Molecule Definition List are correct, click the

Open Goal List button () on the Main Toolbar. This opens the Lists dialog box (Fig. 10.6), where the two fragments are listed in the Fragments section.

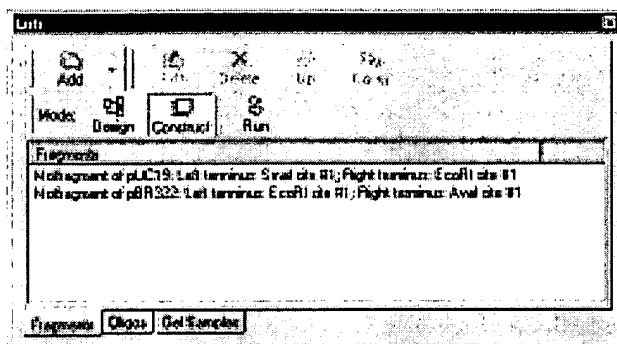


Fig. 10. 6 Lists dialog box

7. Enter General Information About the New Molecule

Press the **Run** button. Click in the Name field text box in the Construct Molecule dialog box (Fig. 10.7) and enter the name of the new molecule, **TUTORIAL1**.

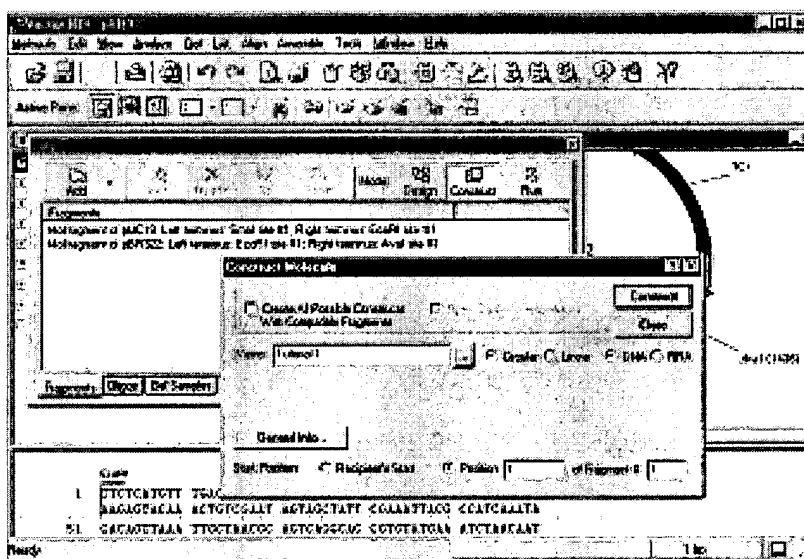


Fig. 10. 7 Construct Molecule dialog box

The radio buttons on the bottom describe the choices for the new molecule start. The first fragment in the list of component fragments is always considered the “recipient” molecule. Click the **Recipient's Start** button to position the start of the new molecule at the same place where pUC19 starts. (If you prefer, you can choose any nucleotide of any component fragment as the starting coordinate of the new molecule by checking the **Position ... of Fragment # ...** button. By default, it's the first nucleotide of the first fragment.

Press the **General Info** button, opening the General Data dialog box where you can specify general information about the new molecule (Fig. 10.8).

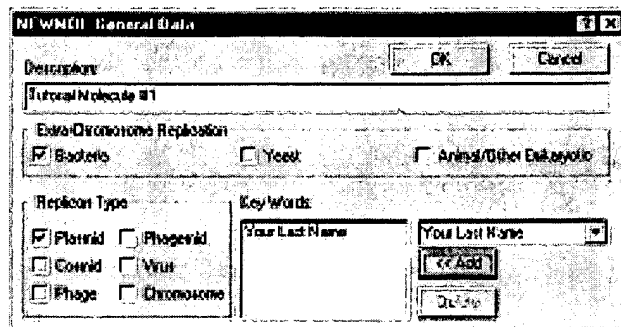


Fig. 10. 8 General Data dialog box, where you can specify general information about the new molecule

- Click in the Description field and type *Tutorial molecule #1*.
- In the Extra-Chromosome Replication field, check **Bacteria**.
- In the Replicon Type field, select **Plasmid**.
- In the text entry box of the Key Word area, enter your last name. Press the **Add** button to add your last name to the list of keywords for this molecule. *Keywords are a convenient way to find molecules you created—just search Vector NTI's database for all molecules with your last name as a keyword.*
- Press **OK** to return to the Construct Molecule dialog box.

8. Attempt to Construct the New Molecule

Press the **Construct** button in the upper right corner of the Construct Molecule dialog box. In the Insert Molecule into Main subbase dialog box, name your subbase **Tutorial** and confirm the creation of the subbase. Click **OK**. Vector NTI attempts to construct your new molecule and save it into the database. Vector NTI soon informs you that the left end of fragment #1 (the *Sma*I site) is incompatible with the right end of fragment #2 (the *Ava*I site).

VNTI cannot process your molecule. Because you are constructing a molecule in which you have defined the fragments, you try to figure out why your fragments are incompatible. If you were to check the fragment termini, you would learn that the blunt 5' end of the pUC19 fragment can't link with the cohesive 3' end of the pBR322 fragment. You need to modify these termini to make them compatible.

Click **OK** in message dialog boxes to return to the Construct Molecule dialog box.

9. Fill in the *Ava* Site of the pBR322 Fragment to Make it Blunt

Close the Construct Molecule dialog box to return to the Lists dialog box. In the Fragments section, click on the second fragment in the list ("Molfragment of pBR322"). As you make the selection, the **Edit** button becomes enabled (Fig. 10.9).

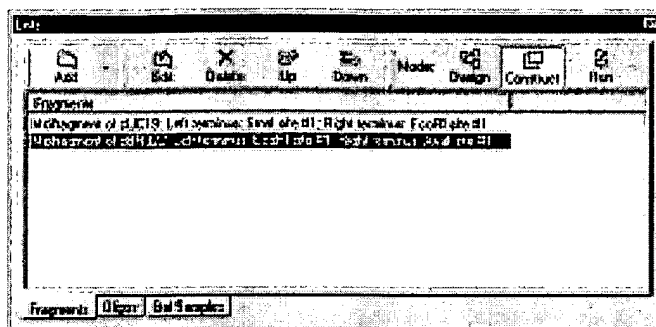


Fig. 10. 9 Enabling the Edit button in the Lists dialog box

Press the **Edit** button, opening the Fragment Editor dialog box (Fig. 10.10).

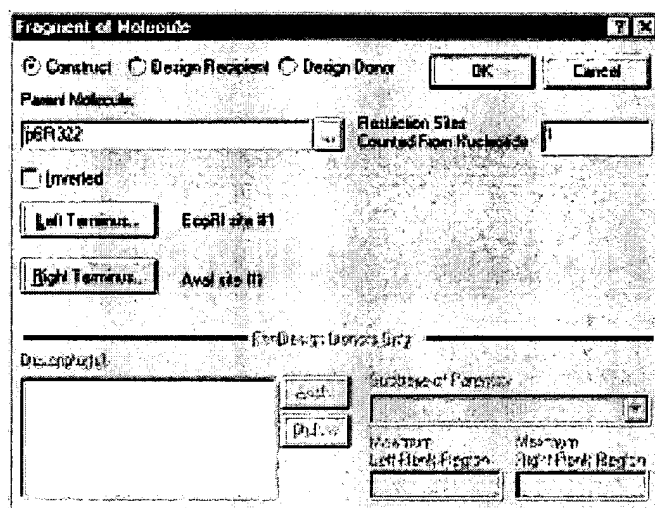


Fig. 10. 10 Fragment Editor dialog box

Click on the **Right Terminus** button, because you need to modify the *Ava*I site that makes up this fragment's right terminus. The Terminus Editor dialog box opens (Fig. 10.11).

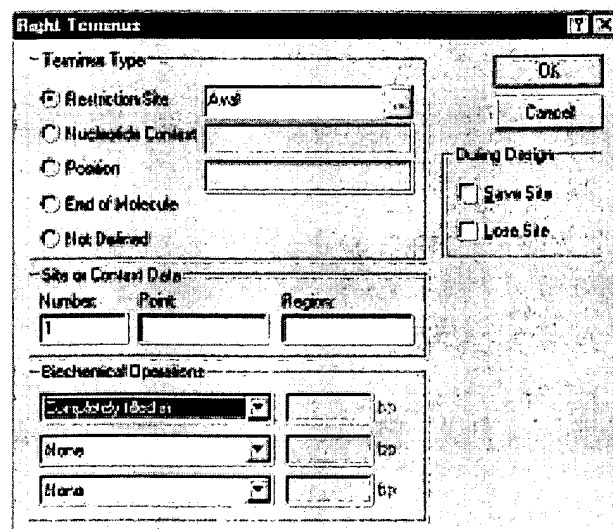


Fig. 10.11 Terminus Editor dialog box

Because you need to perform a biochemical “operation” on the ends of the fragment, in the Terminus Editor, look in the Biochemical Operations section in the lower left corner. Although VNTI can handle up to 3 sequential biochemical operations per terminus, you need to perform only one, filling in the cohesive Aval site. Click in the first biochemical operations box. From the list of allowable options, choose **Completely Filled In**. Then click **OK** in the Terminus Editor and **OK** in the Fragment Editor, returning you to the List dialog box

Press the **Run** button on the Lists dialog box to launch the Construct Molecule dialog box. Press the **Construct** button. Press **OK** to confirm that the “Tutorial” subbase should be used. In the new dialog box, press the **Overwrite** button to overwrite your previously saved TUTORIAL1. Vector NTI analyzes your molecule and this time enters it into the database.

10. Inspect Your New Molecule

After creating the new molecule and saving it to the database, a new Molecule Display window opens with your new molecule. Find TC(R) on TUTORIAL1's graphical map. The molecule's functional and restriction maps are created them automatically. *Vector NTI's is able to create the maps and nucleotide sequences for new molecules with complete accuracy, even in the most complicated cloning situations.*

Chapter 11 Tutorial: Molecule Design

Introduction

This chapter introduces you to the creation of a new molecule by *Design*, Vector NTI's term for using its built-in biological knowledge for new molecule creation. In *Design*, the user defines the molecule fragments, but the restriction sites, methods of terminus modification, etc., are left up to Vector NTI. The *Design* process results in a construction plan which takes advantage of the best possible restriction sites and recombinant strategy.

At the end of this tutorial session, you will be able to:

- Define the recipient and donor fragments for *Design*, using the Fragment Wizard
- Confirm the correct order of recipient and donor fragments in the Design Molecule dialog box
- Name and describe the new molecule
- Specify *Design* parameters and preferences
- Direct Vector NTI to design the new molecule

Fragments are described as they were for molecule construction. Fragments for *Design*, however, must consist of exactly one recipient fragment from the parent molecule, which must be listed first in the Goal Molecule Definition List, and one or more donor fragments. Every nucleotide in the designated recipient fragment is included in the final molecule. Vector NTI searches the parent molecule nucleotides outside of the recipient fragment to try to find convenient restriction sites for inserting donor fragment(s).

You may want to indicate a specific restriction site at one or both ends of the recipient fragment. If a terminus of the recipient fragment is a restriction site, then Vector NTI automatically uses that restriction site in building the new molecule. You may request to save or lose such a specific restriction site. If it is not specified that the site should be retained, then that site may disappear from the final created molecule even if you did not specify that the site must be lost.

Fragments other than that listed first on the list are called donor fragments. Because the purpose of donor fragments is to carry functional signals into the recipient, you should describe a donor fragment, not by its termini, but by the functional signals it contains. Thus when you define a donor fragment in a Molecule Display window, you should click on specific functional signals. Every nucleotide of these specific functional signals is included in the goal molecule, but nucleotides outside these functional signals may or may not be included in the goal molecule. Without at least one functional signal, a fragment cannot be treated as a donor fragment.

A donor fragment can contain flanking regions outside the functional signals. In describing a donor fragment, if you drag the edges of the selection zone beyond the functional signals you have targeted, the fragment will be defined as the functional signal(s) plus flanking

nucleotides. You have defined the maximum allowable flanking regions. Vector NTI tries to reduce the flanking regions of donor fragments to be as small as possible, but does not go outside of these flanking regions when looking for restriction sites.

In this exercise you will perform essentially the same cloning procedure as you did in the Construction tutorial. This time, however, you will simply define the fragments and allow Vector NTI to design the rest.

Follow the steps of the tutorial in the order shown. Figures show what your screen should look like at various points along the way.

1. Launch Vector NTI and Create Display Windows for pbr322 and puc19

Launch Vector NTI using techniques you learned in previous tutorials. Open Molecule Display windows containing the molecules pBR322 and pUC19.

2. Arrange Display Windows

Do not maximize each window this time, but select **Window > Tile Vertical**. The display screen for both molecules appears at the same time, making it easy to work with them side by side (Fig. 11.1). Activate either window (indicated by a dark blue title bar) with a left click. Using the split bars, reduce the Text and Sequence Panes for each molecule to optimize viewing the molecule maps. Use the shift + **Zoom** buttons to arrange the panes so you can easily read the features and labels.

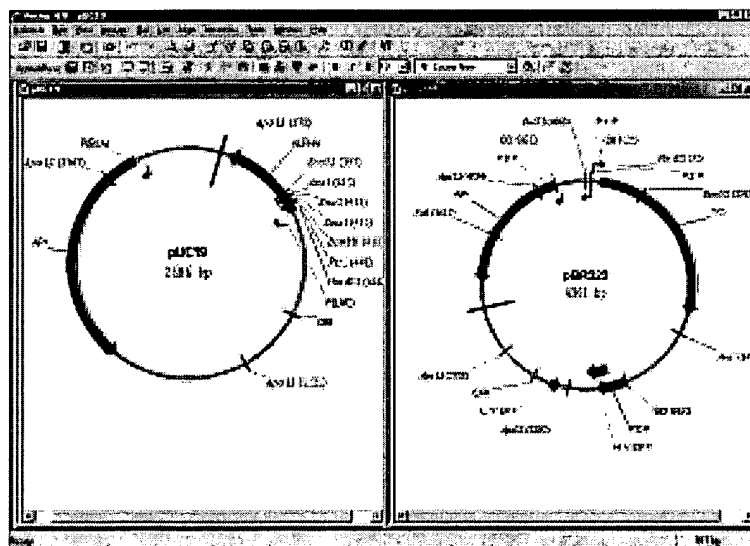


Fig. 11.1 Displaying two screens using Tile Vertical feature

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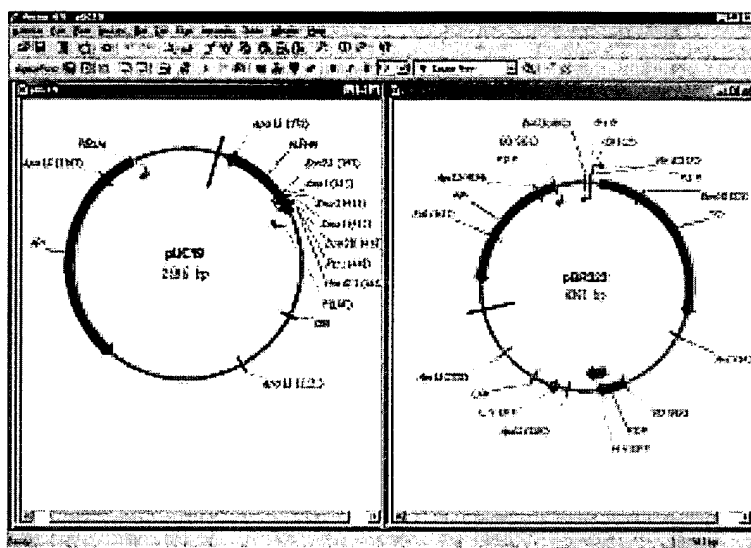



Fig. 11.1 Displaying two screens using Tile Vertical feature

3. Define the Recipient Fragment (from pUC19)

For the goal molecule, the recipient fragment is most of the pUC19 molecule. (Molecule pBR322 provides the donor fragment.) In the pUC19 Display window, activate the Graphics Pane. Press the **Add Fragment to Goal List** button () , opening the Fragment Wizard. You learned in the previous tutorial that Fragment Wizard guides you through the process fragment selection.

- **First screen:** Select the **Design Recipient Fragment** option and click **Next**.
- **Second screen:** For the 5' terminus of the new fragment, in the **Set To A Position** option, enter **500** in the text box. Click **Next**.
- **Third screen:** Enter **250** in the **Set to a Position** text box to define the 3' terminus. Click the **Finish** button to complete the definition of the recipient fragment.

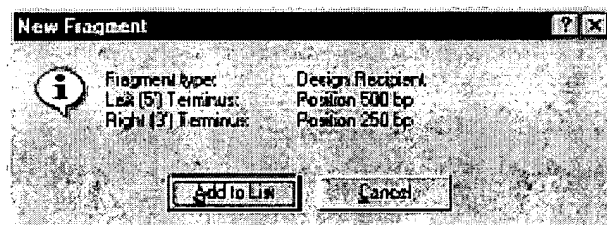




Fig. 11. 2 New Fragment message box

In the New Fragment message box, check the description of the fragment (Fig. 11.2). If correct, press the **Add to List** button, adding the recipient fragment to the Molecule Goal list.

In the Graphics Pane, the recipient fragment is marked with a wireframe. Note that the polylinker near the 2 o'clock position is *outside* the selection. If you included the polylinker in the selection, Vector NTI would not be able to use those restriction sites because *every nucleotide included within the selection zone in the recipient fragment will be included in the final molecule*.

4. Define the Donor Fragment (from pBR322)

In the pBR322Display window, activate the Graphics Pane, then press the **Add Fragment to Molecule Goal List** button () opening Fragment Wizard again.

- **First screen:** Select the **Design Donor Fragment** radio button. Click **Next**.
- **Second screen:** Move the cursor until it is over the TC(R) arrow or label in the Graphics Pane. *Drag the Fragment Wizard out of the way, if necessary*. The cursor changes to a hand () , indicating that you are pointing directly at a functional signal. Click once to select the entire TC(R) signal. The selection wireframe appears, aligned

directly with the start and end of TC(R). The name of the signal is displayed in the Fragment Wizard.

Click the **Finish** button. The default values are used to complete the definition of the fragment. Continuing with the Fragment Wizard allows specifications for more complicated design, to be covered in the next chapter.

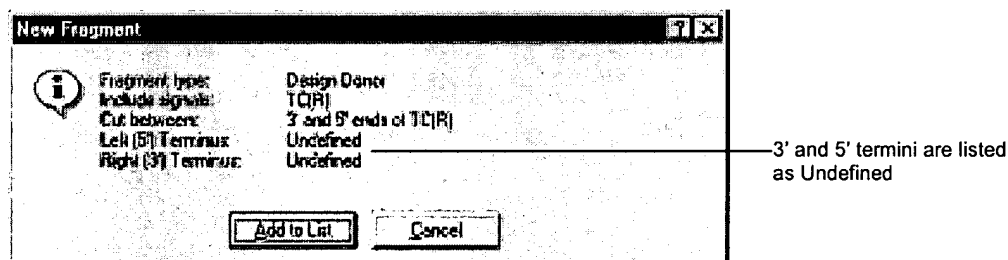


Fig. 11.3 New Fragment message box, with 3' and 5' termini of the donor fragment described as "Undefined"

In the New Fragment message box, check the donor fragment description. Note that the 3' and 5' termini of the donor fragment are described (correctly) as "Undefined" (Fig. 11.3). Press the **Add to List** button, adding the donor fragment to the Molecule Goal list.

5. Inspect the Goal Molecule Definition List

Press the **Open Goal List** button (img alt="Open Goal List icon: a square with a magnifying glass over a list") in the main toolbar, opening the Lists dialog box. Notice that the **Design** button is selected, indicating that Vector NTI already has recognized that your fragments are for Design and not Construction.

In the Goal Molecule Definition List appearing in the Fragments section of the dialog box, inspect the two fragments you defined. For the recipient fragment, (pUC 19, listed first,) the termini are defined by nucleotide positions. For the donor fragment (pBR322, listed second), the termini are not defined, (NODEF), but the fragment must contain the TC(R) descriptor.

Note: For Design to proceed correctly, recipient and donor fragments must be listed correctly. If one is listed incorrectly, select it and use the **Up** and **Down** buttons to reposition it.

6. Enter General Information for Your New Molecule

Press the **Run** button. Although this box is almost identical to the Construct Molecule dialog box, in the previous chapter, its name and operational mode have changed to Design Molecule. This is a result of the molecule building process you designated in the Fragment Wizard.

In the Name text box, enter the name of the molecule, **TUTORIAL2**. Press the **General Info** button. In the General Data dialog box, in the Description field, enter **Tutorial molecule #2**.

Set the Extra-Chromosome Replication type to **Bacteria** and the Replicon Type to **Plasmid**. To enter your last name as a keyword, select it from the existing keyword list in the drop-down menu and press the **Add** button.

Press **OK** to return to the Design Molecule dialog box (Fig. 11.4).

Check the **Recipient's Start** button in the radio button group on the bottom of the Design Molecule dialog box to position the start of the new molecule at the same place where the recipient molecule (pUC19) starts.

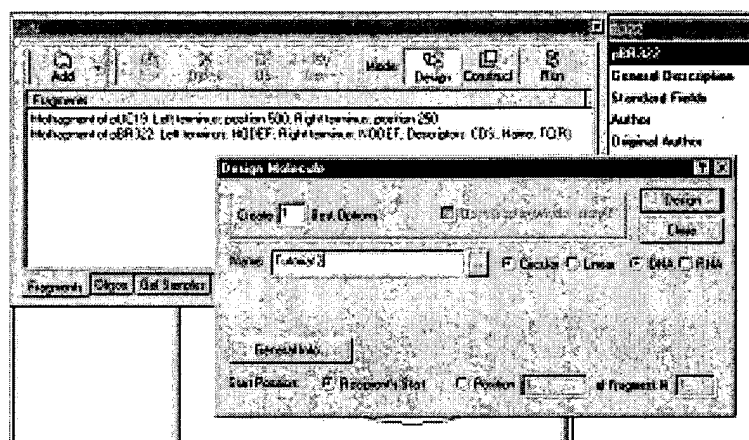


Fig. 11. 4 Entering general information for your new molecule in the Design Molecule dialog box

7. Prepare to Design the New Molecule

Press the **Design** button in the upper right corner of the Design Molecule dialog box. When asked, select the "Tutorial" subbase you created in the last tutorial, and press **OK** to continue. The Design Parameters dialog box opens (Fig. 11.5):

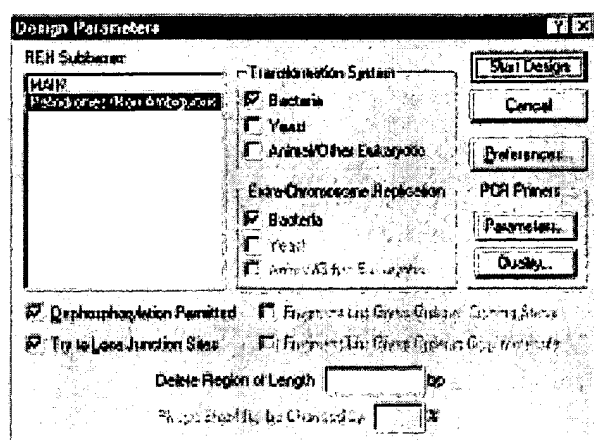


Fig. 11. 5 Design Parameters dialog box

Here you can select the subbases of restriction endonucleases (RENs) that Vector NTI considers when designing the new molecule. For example, you could create a subbase listing the RENs you have on hand in the laboratory and tell Vector NTI to design the new molecule using only those enzymes.

You can also indicate the transformation systems to be used in your experiments as well as the presence or lack of extra-chromosome replication capability of your molecule in those transformation systems. You can permit or forbid the use of dephosphorylation as a preselection method, etc.

Make sure that the Palindromes/Non-Ambiguous REN subbase is selected. For this tutorial, leave all other parameters at their default values.

8. Set Your Preferences for Molecule Design

Now click on the **Preferences** button, opening the Design Preferences dialog box (Fig. 11.6) In this box, you can choose the parameters *you* prefer to create new molecules. You can designate which genetic engineering techniques are permissible and assign priorities for use of the permissible techniques. You can set preferences in the categories of fragment isolation, fragment ligation, and terminus modification.

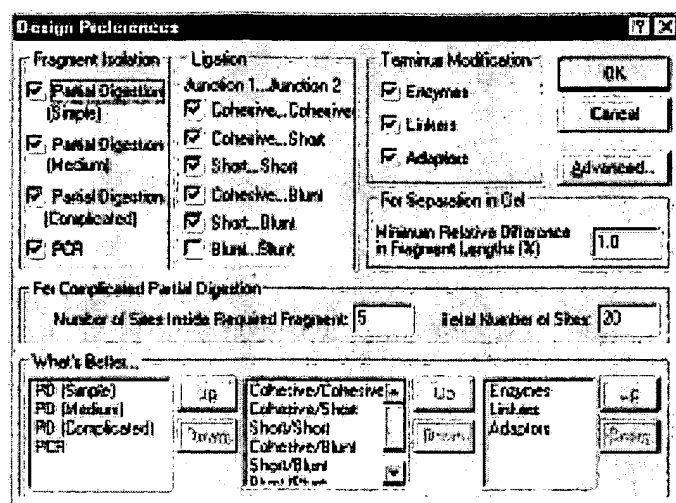


Fig. 11. 6 Design Preferences dialog box, where you can choose the parameters you prefer to create new molecules

You may find it useful for Vector NTI to design the same goal molecule several times, changing the design preferences each time. This gives you several alternatives for constructing your molecule.

Let's change the preferences for fragment ligation: Turn off the check box for blunt–blunt ligation. In blunt–blunt ligation, both the donor and the recipient have only blunt termini. Since we are deactivating this option, Vector NTI will make sure that all fragments have at least one cohesive end.

Below the check boxes are priority lists specifying which of the techniques are preferable. Leave these at their default values.

With the **Advanced Preferences**, accessible through the Design Preferences dialog box, you could alter the way Vector NTI's evaluates possible design paths. For this tutorial, ignore the Advanced Design Settings.

Press the **OK** button, accepting the Design Preferences and returning to the Design Parameters dialog box.

9. Design the New Molecule

Press the **Start Design** button. Vector NTI generates many possible ways of cloning the donor into the recipient and seeks the best solution using its biological knowledge and the preferences you indicated. An optimum cloning method is quickly found and the goal molecule is created based on that best option.

10. Inspect the New Molecule

When the design is complete Vector NTI opens a new Molecule Display window containing the molecule you have created (Figure 11.7).

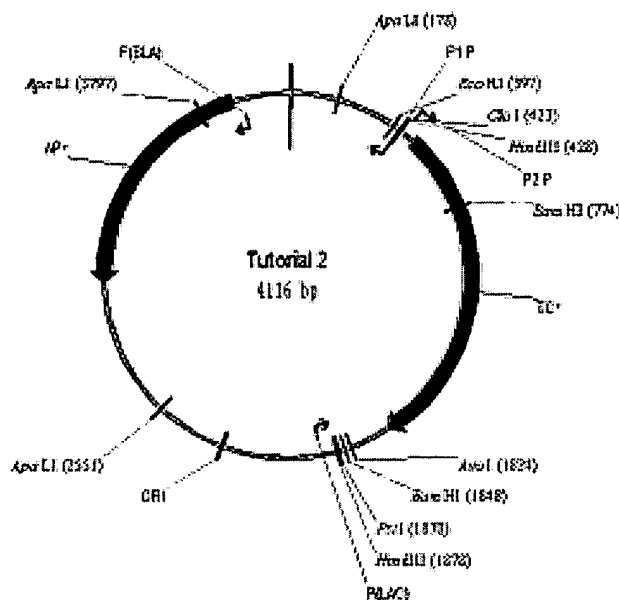


Fig. 11. 7 Inspecting the new molecule you have created

Maximize and inspect the graphical map and text description of your new molecule. In the Text Pane, the new Design Description folder contains instructions for creating TUTORIAL2 at the laboratory bench.

11. Inspect the Design Plan

In the Text Pane, open the Design Description folder and its subfolder labeled Step #1.

- Vector NTI uses the *Sma*I and *Eco*RI sites of the recipient, and the *Eco*RI and *Mlu*NI sites of the donor. *The donor fragment has short flanking regions on either side of the functional signal TC(R).* The sites chosen are compatible, so no biochemical operations are necessary to modify the termini.
- With the selected restriction sites, each fragment has one cohesive end and one blunt end, so blunt–blunt ligation is avoided as requested. The selected cloning option gives the required orientation of the cloned fragment in the recipient.
- One of the recipient's restriction sites—*Sma*I—is lost after ligation. This allows preselection of ligated molecules using *Sma*I before transformation. Because the recipient's *Sma*I site is lost in the successful cloning process, this prescreening eliminates improperly ligated fragments. As only one transformation system (bacteria)


was designated, Vector NTI suggests no alternatives. If you had permitted several transformation systems, Vector NTI would have chosen the system that involves the least effort.

- For clone analysis after transformation, Vector NTI suggests using the restriction enzyme *BspDI*. Its recognition site is new in the recombinant molecule and does not exist in the recipient.
- For alternative methods of clone analysis, the system recommends an oligonucleotide for colony hybridization and PCR primers to use in that technique.
- Vector NTI lists restriction sites close to the cloned fragment that can be used to isolate the cloned fragment from the recombinant molecule: *EcoRI* and *XbaI*. (*SmaI* and *MluNI* have disappeared in the new molecule.) *Vector NTI has only considered the restriction endonucleases in the REN subbase selected in the Design Parameters dialog box.*
- The Design Description folder shows what sites the recombinant lacks, and lists all unique sites on the recombinant, divided into two groups, those inside the cloned fragment and those outside.

If the design were to consist of more than one cloning step, similar information would be given in a separate folder for each stage of the process.

12. Print Out the Design Plan

To print the design plan generated for your new molecule, activate the Text Pane of the TUTORIAL2 Display window. Close all of the folders, then open the Design Description

folder and the Step #1 subfolder. Press the **Print** button () on the Window Toolbar, and the open folders in the Text Pane are printed to your printer. You now have a hard copy of the Design Plan for TUTORIAL2.

Chapter 12 Tutorial: Advanced Molecule Design

Introduction

In the previous two tutorials, you were introduced to simple molecule creation using Vector NTI's Construction and Design tools. This chapter introduces you to more complex Design procedures. In the first scenario, you will assign some complex conditions to the recipient molecule, leaving the donor fragment relatively simple. In the second, you will use a relatively simple recipient but make the donor fragment more complex. Since you are probably getting tired of pBR322 and pUC19, let's change molecules. You will now use BPV1 and SV40.

At the end of this tutorial session, you will be able to:

- Define the recipient and donor fragments for Design using Fragment Wizard, placing complex requirements upon the recipient.
- Define the recipient and donor fragments for Design using Fragment Wizard, placing complex requirements upon the donor.
- Describe the new molecule
- Design the new molecule.

Follow the steps in the order shown. Figures show what your screen should look like at various points along the way.

1. Launch Vector NTI, Open and Arrange Display Windows


Launch Vector NTI using techniques you learned in previous tutorials.

Using techniques you learned in previous tutorials, open Molecule Display windows for molecules BPV1 and SV40. Select **Window > Tile Vertical** to work with the molecules side by side. Since you will be working with the molecules' graphical maps, arrange the display windows conveniently.

First Design: Complicated Recipient

In the first complex design, you will insert SV40's LARGE_T gene into the second *Apa*LI site of BPV1. You will direct Vector NTI to save the 5' *Apa*LI site and prohibit blunt-blunt fragments. If the donor has *Apa*LI sites appropriate for cutting the cloned fragment, then the problem will be simple. If not, the system must take a more complicated approach to perform the insertion.

2. Define the Recipient Fragment

In the BPV1 Display window, activate the Graphics Pane. Open the Fragment Wizard using the **Add Fragment to Goal List** button () as you did in the previous tutorials, and follow these steps:

- **First screen:** Select the Design Recipient option. Press the **Next** button.
- **Second screen:** To define 5' terminus, click on the label of the *Apa*LI restriction site #2 in the Graphics Pane (nucleotide 7631, or about the 11 o'clock position). Click the **Next** button to continue.
- **Third screen:** Select **Save Site** to save the *Apa*LI site on 5' end of the recipient fragment. Press the **Next** button.
- **Fourth screen:** To define the 3' terminus, press SHIFT + CLICK on the same *Apa*LI site. The name and the position of the site are now displayed in the Fragment Wizard. Press the **Finish** button to complete the definition of the recipient fragment.

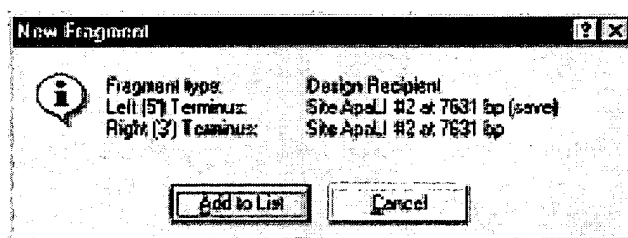


Fig. 12.1 New Fragment message box, with 3' and 5' termini of the recipient fragment set on the same ApaLI site

In the New Fragment message box, inspect the recipient fragment. Note that the 3' and 5' termini of the recipient fragment are set on the same *Apa*LI site (Fig. 12.1). Press the **Add to List** button, adding the recipient fragment to the Molecule Goal list.

3. Define the Donor Fragment

Switch to the SV40 Display window and activate its Graphics Pane. Open the Fragment Wizard again:

- **First screen:** Select the Design Donor option on the first screen; press the **Next** button.
- **Second screen:** Click on the LARGE_T signal's symbol or label in the Graphics Pane to select it. Press the **Finish** button in the Fragment Wizard.

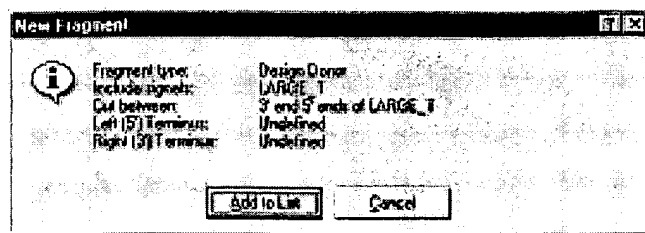



Fig. 12. 2 Added donor fragment to the Molecule Goal List

Inspect the donor fragment in the New Fragment message box, and press **Add to List** button. (Fig. 12.2) The donor fragment is added to the Molecule Goal List.

4. Inspect the Goal Molecule Definition List

Press the **Open Goal List** button () in the main toolbar, opening the Lists dialog box. The Fragments section of the dialog box contains the Goal Molecule Definition List, listing the two fragments you defined. The recipient fragment (BPVI) must be the first in the Goal Molecule Definition List. (If it is not, select the fragment, then click the **Up** button to move it into the correct position.)

Highlight the SV40 fragment and press the **Edit** button, opening the Fragment Editor dialog box. Click the **Inverted** check box to change LARGE_T's direction to match the recipient's direction and press **OK**.

You could leave LARGE_T in its original orientation if you want to; the system will design your new molecule either way. We have changed LARGE_T to Inverted only to demonstrate that Vector NTI can clone fragments in different orientations.

5. Enter General Information for Your New Molecule

Press the **Run** button. Enter the name and description for your new molecule as you learned in previous tutorials:

- Name the molecule TUTORIAL3.
- Press the **General Info** button, opening the General data dialog box:
- In the Description field, enter Tutorial molecule #3.
- Set the Replicon Type to Plasmid,
- Turn on the Bacteria Extra-Chromosome Replication option.
- Enter your name as a keyword.
- Press OK to return to the Design Molecule dialog box.

- Check the **Recipient's Start** button in the radio button group just above the Component Fragments section to position the new molecule start at the same place (if possible) where the recipient molecule (BPV1) starts.

6. Prepare to Design the New Molecule

Press the **Design** button in the upper right corner of the Design Molecule dialog box. When asked for a subbase name, select the **Tutorial** subbase you created before; press **OK** to continue.

The Design Parameters dialog box appears. Leave all the settings at their default values and move on to the next step.

7. Set the Design Preferences

Click on the **Preferences** button, opening the Design Preferences dialog box. Note that the blunt–blunt ligation box is already turned off. Vector NTI remembers your previous design preferences so that you do not have to set them every time you design a new molecule.

Below the check boxes are priority lists specifying which of the techniques are preferable. Leave these at their default values.

Press the **OK** button to accept the Design Preferences and return to the Design Parameters dialog box.

8. Design the New Molecule

Press the **Start Design** button. As before, an optimum cloning method is quickly found, and the goal molecule is constructed based on that best option.

9. Inspect the New Molecule

When the design is complete Vector NTI opens a new Molecule Display window containing the molecule you have created (Fig. 12.3).

design description, recommending that the *AvrII* and *ApaI* restriction sites would allow you to distinguish correct and parasitic orientations in gel.

You may print out this design plan if you wish, and then proceed to the next design task.

Second Design: Complex Donor Fragment

You will now design a second molecule based on BPV1 and SV40, this time making the donor more complicated rather than the recipient.

1. Define Recipient


Return to the BPV1 Display window's Graphics Pane and open the Fragment Wizard:

- **First screen:** Select the Design Recipient option and press the **Next** button.
- **Second screen:** Select the Set to a Position option and enter 5000 as the position of the 5' terminus. Press the **Next** button.
- **Third screen:** To define the 3' terminus, select the Set to a Position option again; enter 2500. Press the **Finish** button.

Inspect the results in the message box, and press the **Add to List** button. The recipient fragment is added to the Molecule Goal List.

2. Define Donor

Switch to the SV40 Display window's Graphics Pane and open the Fragment Wizard.

- **First screen:** Select the Design Donor option and press the **Next** button.
- **Second screen:** Click on the *LARGE_T* signal's symbol or label in the Graphics Pane to select it. Press the **Next** button.
- **Third screen:** The default option on the next screen is "Leave terminus Undefined". Do not change this option; press the **Next** button.
- **Fourth screen:** On this flank region screen, you can specify the maximum size of the flank region or let Vector NTI use all available space outside the selected signal. Select the **Use flank region no larger than** option. You can either enter the maximum length in the text box or select the fragment in the Graphics Pane. Move your cursor over the 5' end of the selection wireframe in the Graphics Pane (indicated by the  symbol). Click and drag the 5' end to the position at about nucleotide 2250. *The current position is displayed in the status bar.* (Remember you can use shift + right and left arrow keys to fine-tune the selection.) The edit box in the Fragment Wizard shows you the maximum size of the flank region (it should be about 400 nucleotides). When you are finished, press the **Next** button to go to the 3' terminus screen.
- **Fifth screen:** Specify here that Vector NTI should use the *NcoI* restriction site at nucleotide 38 to cut the 3' terminus of the donor fragment. Select the **Use specific site** option, SHIFT + CLICK on the *NcoI* restriction site at nucleotide 38. (Drag the Fragment Wizard out of the way, if necessary). The name and the position of the

restriction site appear in the Fragment wizard screen. Press the **Finish** button to complete the definition of the donor fragment.

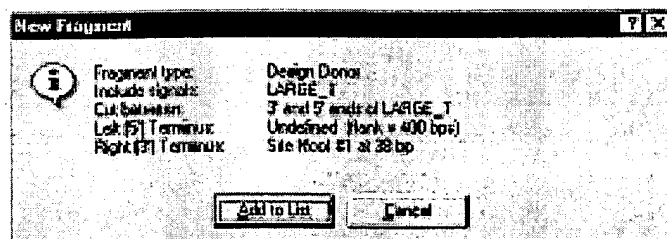


Fig. 12. 4 New Fragment message box, with 5' terminus described as "Undefined," specified maximum length of a flank region, and 3' terminus set to the NcoI site

In the New Fragment message box, inspect the selected donor fragment. Note that the 5' terminus is described as "Undefined," with the specified maximum length of a flank region and the 3' terminus is set to the NcoI site (Fig. 12.4). Press the **Add to List** button, adding the donor fragment to the Molecule Goal list.

3. Inspect the Goal List

Click the **Open Goal List** button to open the List dialog box and inspect the fragments you have defined. The recipient fragment (BPV1) must be listed first in the Goal Molecule Definition List. Double-click the donor fragment, opening the Fragment Editor. One end of the donor is determined by a restriction site, while the other end has a flank region defined. This makes the situation considerably more complicated than our previous cases, where the donors were more simply defined. Click the **Inverted** check box and press **OK**.

4. Design the New Molecule

Press the **Run** button. Enter the name **TUTORIAL4** and enter the other necessary information about the new molecule. Check the **Recipient's Start** button to make the start of the new molecule at the same place (if possible) where the recipient molecule (BPV1) starts.

Press the **Design** button in the upper right corner of the dialog box. Select the "Tutorial" subbase for the molecule and press **OK** to continue. Leave the Design Preferences at their default settings and press the **Start Design** button. After a few moments, Vector NTI produces a workable design for the molecule you have requested.

5. Inspect and Print the New Molecule

In the new Molecule Display window containing TUTORIAL4, inspect the Step #1 subfolder of the Design Description folder. In this case, despite the more complex conditions you specified, Vector NTI found a simple and convenient design. Both donor and recipient can be isolated at the NcoI and BamHI sites, so the cloning is straightforward.

You specified a maximum flank region of about 400 nucleotides; Vector NTI found a good *Bam*HI site, allowing the actual flank region to stay within the assigned limit.

If you wish, print out the design description and/or graphical map of TUTORIAL4.

Chapter 14 Tutorial: Internet Connectivity and Tools

Introduction

This chapter introduces you to Vector NTI tools that provide connections to external programs and Internet services.


At the end of this tutorial session, you will be able to:

- Send data from Vector NTI to several public WWW servers
- Perform a simple BLAST search, alignment and analysis
- Save Internet search results as Comments in a database file

Follow the steps in the order shown. Figures show what your screen should look like at various points along the way.

1. Launch Vector NTI and Open pBR322 in a New Display Window

Launch Vector NTI by double-clicking its icon in the program group or folder in which you

installed Vector NTI. Click the **Open** button () on the main toolbar. Open the Molecule Viewer for pBR322 using the techniques you learned in previous tutorials. Maximize the Molecule Display window.

2. Select the Whole Sequence of pBR322 and Use the BLAST Search Tool

Select **Tools > Compare Against > GenBank via BLAST on NCBI Server**.

Note: Some World Wide Web browsers require the Internet connection to be established before you start the browser. If your TCP/IP stack or other Internet connection software cannot be started “on the fly” you need to connect before selecting any of Vector NTI’s Internet-related commands.

In the Sequence Data dialog box of the BLAST viewer (Fig. 14.1), choose the range and strand of the sequence to be sent to the server: Whole Sequence / Direct Strand.

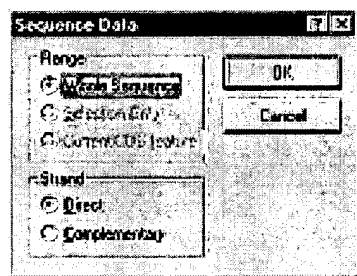


Fig. 14. 1 Sequence Data dialog box of the BLAST viewer

Press **OK** to continue. Your selected browser may open automatically or a list of available browsers may open.

If appropriate, select the name of your WWW browser from the list and press **OK** to continue. This configures Vector NTI's connection to the WWW for all subsequent connections.

Note: The standard Vector NTI distribution contains support modules for many popular WWW browsers. If your WWW browser is not in the list, try selecting one of the "Autodetect" modules from the displayed list. The latest additions to the list are available on the Vector NTI WWW home page: <http://www.informaxinc.com/>

Vector NTI opens the BLAST Search page in the WWW browser (Fig. 14.2). (The page may look only similar to this, depending on your browser).

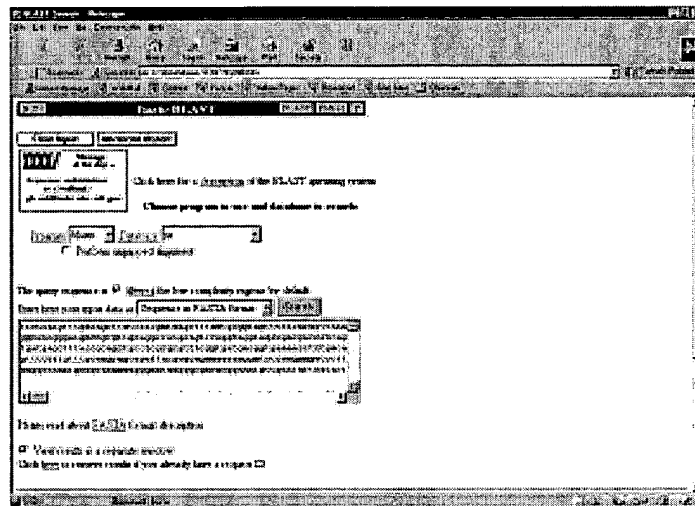


Fig. 14. 2 BLAST Search page in the WWW browser

This page is Vector NTI's gateway to the NCBI BLAST Search server and contains the options that can be used to change various BLAST search parameters. In the Database section select the "vector" database.

The sequence of pBR322 is already entered in the Query Sequence field. Press the **Search** button.

Note: At this step, your WWW browser may display a dialog box or start a program to establish an Internet connection. Enter the required parameters and continue.

Once an Internet connection with the NCBI server has been established, you may receive a screen saying your request has been placed in a BLAST queue. To proceed, click on

Format Results. The response time for the BLAST results varies depending on the server load. If you receive an error, return to the request page and click on the **BLAST search using sequence data** link on the top of the page to get information about BLAST server operation. If the server is busy, try waiting several minutes and resubmit the query.

Note: Since the interface of WWW servers changes from time to time, you may receive a message that the requested resource is not found. This means that Vector NTI's server gateway page is obsolete. The latest versions of Vector NTI gateway pages are available on the Vector NTI WWW home page.

When you receive the results from the BLAST server, click on the second molecule link (at the time of this writing (4/2000), the first hit was pBR322 itself and second hit was U03501/YRP7). Another page appears, containing the GenBank description of the molecule (Fig. 14.3):

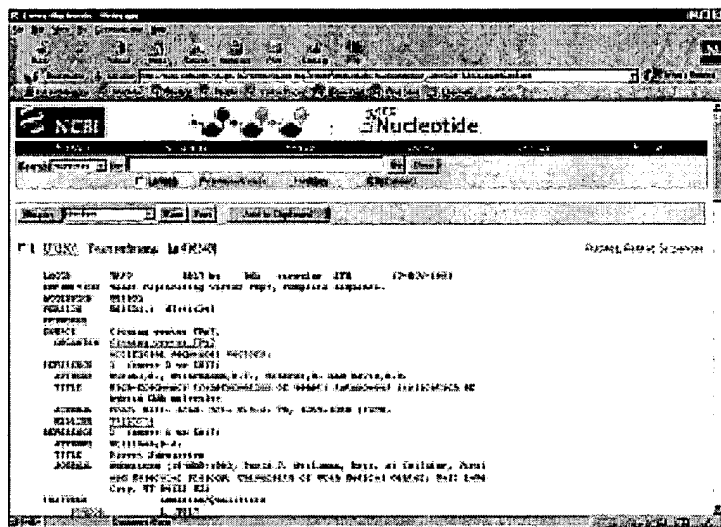


Fig. 14.3 Web page containing the GenBank description of the molecule

3. Display the Result of the Query in a Molecule Display Window

To transfer the result of the query back to Vector NTI, highlight the GenBank text starting at the word “LOCUS” and ending with the “//” at the end of the sequence. Select **Edit > Copy** from the menu bar (or its analog in your browser) to copy the selected text to the clipboard. Switch back to the Vector NTI workspace and select **Tools > Open > DNA-RNA Molecule from Clipboard > in GenBank Format** from the menu bar (Fig. 14.4). Vector NTI opens your molecule in a new Display window, automatically generating the restriction map and graphics representation of the molecule.

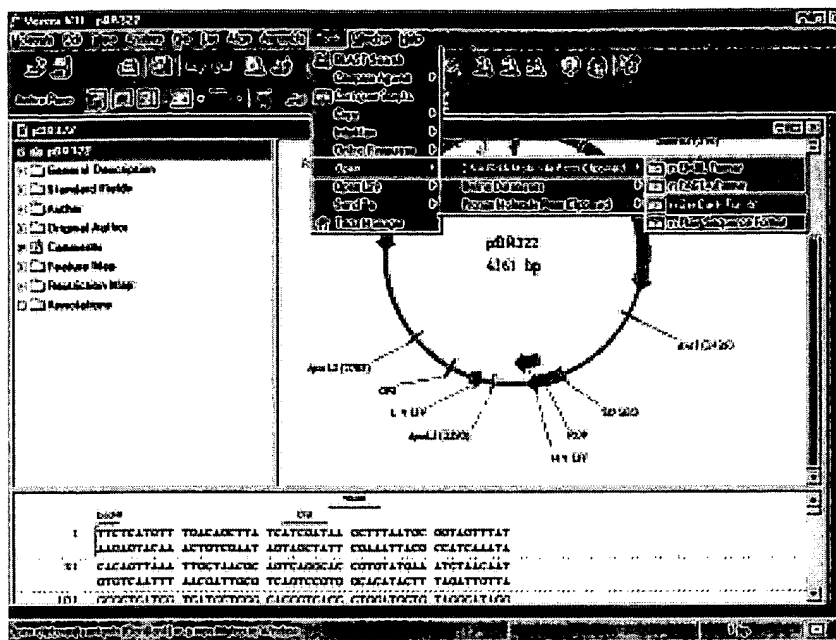


Fig. 14. 4 Transferring the result of the query back to Vector NTI

4. Save or Open the Result of the Query Using the Save Button

An alternative way to transfer search results from the NCBI server to Vector NTI is to use the **Save** button on the query's GenBank report. You can also use the **Save** button to save the query molecule to your hard drive as an independent file.

Switch back to the U03501/YRP7 GenBank report and locate the **Save** button just above the text of the GenBank report. Press the **Save** button. The File Download dialog box appears. If you choose the Open option, your browser will try to load the document into the associated application. If Vector NTI was configured correctly, a new window should appear in its workspace showing the U03501 molecule. If you choose the Save option, you can specify a file name and a location to store the U03501 molecule as a file on your hard drive.

5. Use Alignment and Analysis Tools

The standard distribution of Vector NTI has several tools to perform sequence alignment and analysis. To perform the alignment, switch to the Database Explorer window. (Click the **VNTI Database Explorer** button on the taskbar at the screen bottom, or open it using the

Local Database button () or select **Database > Explore** on the menu bar. Select the Proteins table from the drop-down menu.

pattern matches listed in their numerical and graphical form. For details, click on the site ID links, such as PDOC00001 for the glycosylation site, opened and illustrated in Fig. 14.6.

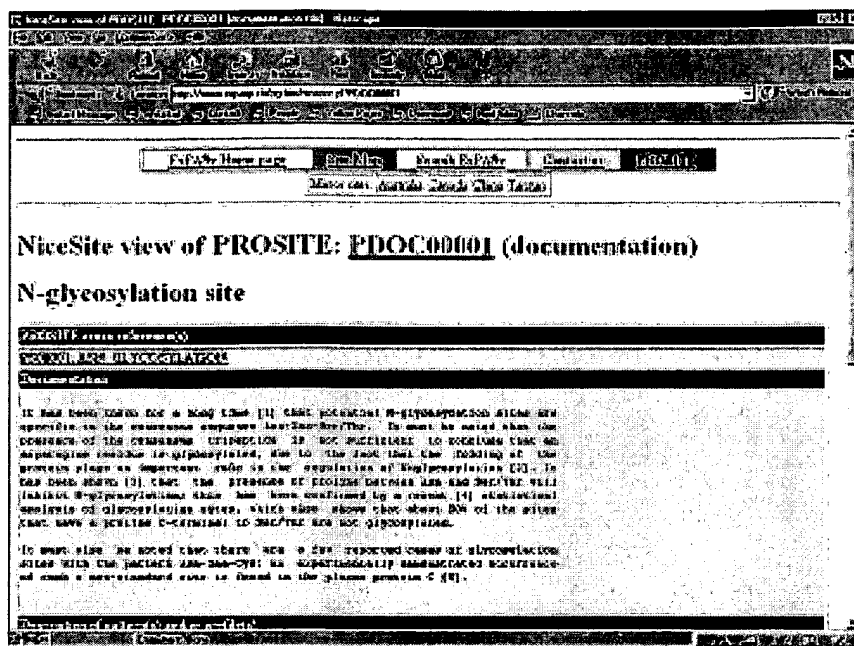


Fig. 14. 6 Site ID link for PDOC00001 (glycosylation site)

6. Saving Search Results as Comments

Vector NTI allows you to associate any text data with database objects in the form of comments. Let's save the search results to the database for future reference.

Select the resulting hits, starting with hit [1], by dragging the mouse across and down the page. Do not include the sequence, which is already stored in the Vector NTI database.

Choose **Edit > Copy** or press CTRL-C to copy results to the Clipboard Return to Database Explorer, and with 41_HUMAN still selected in the Database Explorer window, select **Protein > Edit** from the Explorer's menu bar.

In the tabbed Edit dialog box that opens, you can edit various information associated with the protein molecule (Fig. 14.7). Click on the **Comments** tab, scroll to a point at the end of the existing comment text and click to set the text cursor for insertion.

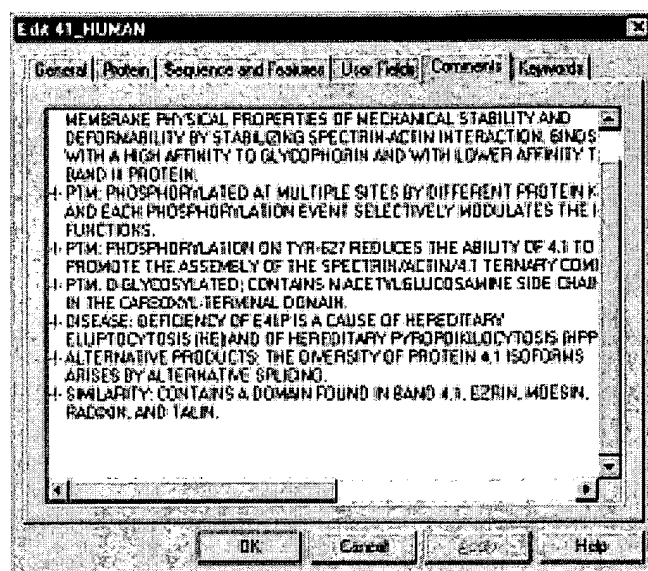


Fig. 14. 7 Edit dialog box, where you can edit information associated with the protein molecule

Now press CTRL-V to paste the search results from the Clipboard.

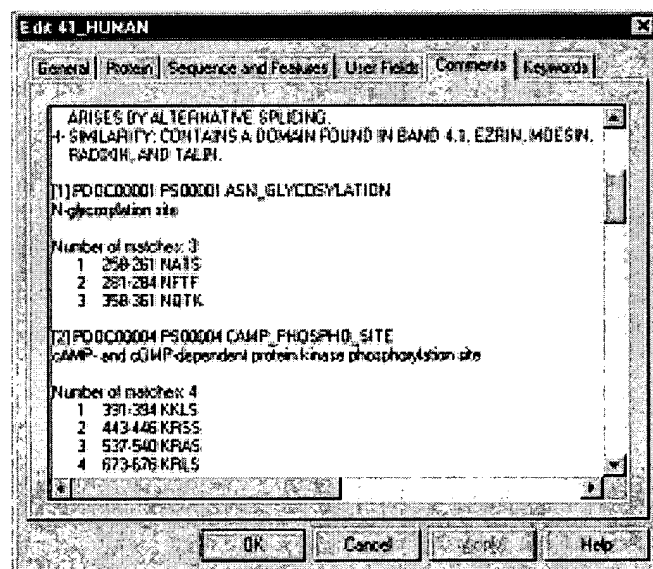


Fig. 14. 8 Editing the resulting text

Edit the resulting text to your liking and press the **OK** button to save it to the database (Fig. 14.8).

The search results are now saved in the database for later retrieval. To view the comments from 4l_HUMAN's Display window, double-click on the Comments line in the Text Pane of a Molecule Display window for 4l_Human.

7. Close the Molecule Display Windows and Exit Vector NTI

You have now taken a first look at Vector NTI's Tools for Internet connection. To close all display windows, select **Molecule > Close**; to exit the program, select **File > Exit** from the menu bar. If you are not going to continue your work with the Internet, close the WWW browser and disconnect, if necessary.

Chapter 15 Tutorial: AlignX

Introduction

This chapter introduces you to operations of AlignX. The simultaneous alignment of many nucleotide or amino acid sequences is an essential tool in molecular biology. Multiple alignments are used to find diagnostic patterns, characterize protein families, as well as to detect or demonstrate a similarity between new sequences and existing families of sequences. They are also useful in predicting secondary and tertiary structures of new sequences, suggesting oligonucleotide primers for PCR and serving as an essential prelude to molecular evolutionary analysis.

At the end of this tutorial session, you will be able to:

- Import molecules from other Vector NTI Suite applications into AlignX
- Describe elements of the AlignX user interface
- Create an alignment, and add molecules to an alignment
- Modify alignment graphics and sequence
- Edit an alignment
- Create and interpret an AlignX Dot Matrix
- Export molecules from AlignX into other Vector NTI applications

Follow the steps of the tutorial in the order shown. Figures show what your screen should look like at various points along the way.

1. Launch AlignX from the Database Explorer

Using techniques you learned in previous tutorials, open the Vector NTI Suite Database Explorer. Now open AlignX by double-clicking its icon in the program group or folder in which you installed Vector NTI. In the Database Explorer, active the Protein table and select the Protein Molecules (MAIN) subbase. In the Database Objects List Pane, scroll to the molecules 5HIA for human, rat, and mouse. Click the cursor at the right bottom of the last of the three proteins, and drag a box around all three molecules. This is a new way to select a group of objects in a subbase list (Fig. 15.1).

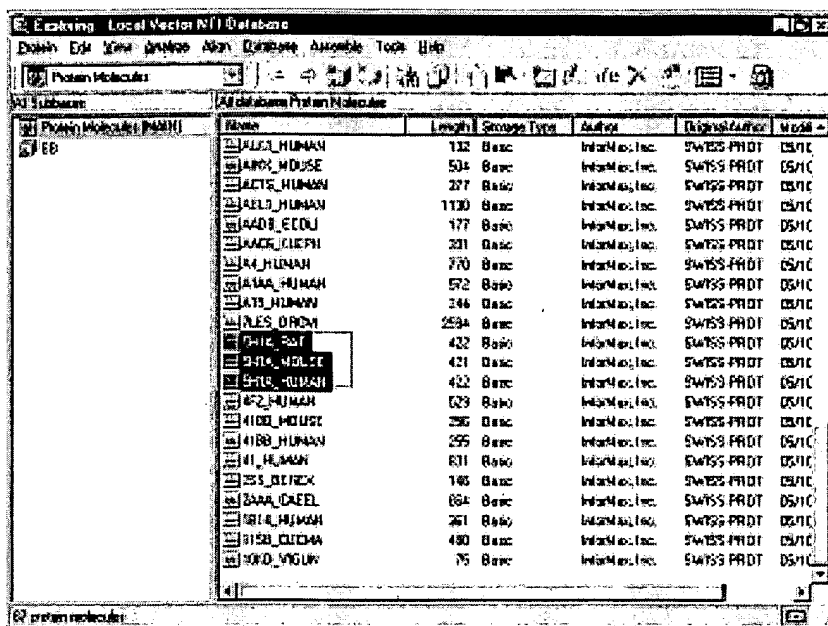



Fig. 15. 1 Selecting a group of objects in a subbase list

Now position the Database Explorer window and the AlignX window side by side, and click and drag the selected molecules into the AlignX viewer. Now in the protein list, also select and drag into AlignX the 41BB Human molecule.

An alternative method is to select the molecules in Database Explorer and select **Align > AlignX – Align Selected Molecules**.

2. Examine AlignX Display Window

When you open AlignX, even after bringing molecules in, three of the panes are empty. After you initiate the alignment, the alignment results will appear in the empty panes.

In the Text Pane, select the 5H1A molecules. Press the **Align** button () , initiating the alignment that now appears in the display window.

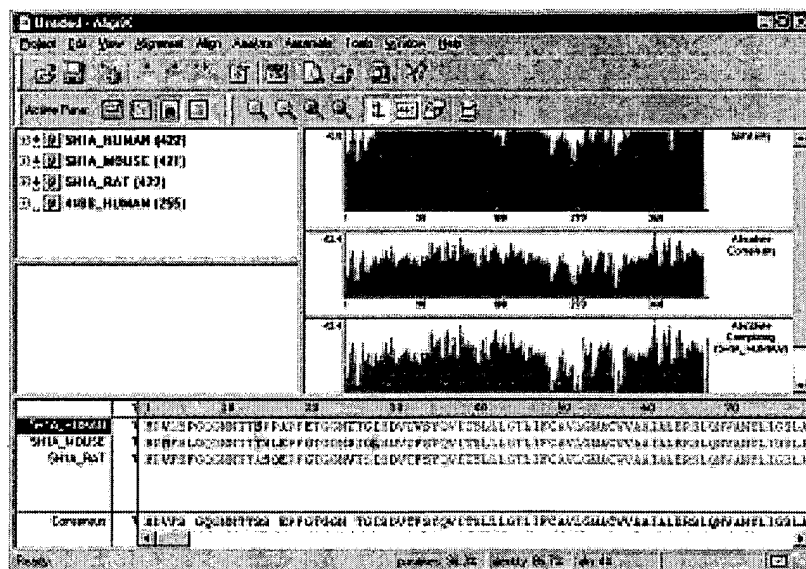



Fig. 15. 2 AlignX Display window

The AlignX Display window consists of a menu bar, three toolbars and context menus specific for each pane (Fig. 15.2). The AlignX Display window includes the Text Pane, the Phylogenetic Tree Pane (still empty), the Analysis Pane and the Alignment Pane. The panes are divided by split bars that can be moved to resize the panes.

All of the toolbar functions are summarized in Chapter 4. Toolbar buttons are described in this tutorial as needed.

As in the other Vector NTI Suite applications, to apply a command from the main menu in a given pane (Print, Print Preview, Camera), that pane must be active. The active pane can be

toggled with the Switch Panes () buttons in the Pane Selection Toolbar or by clicking anywhere in the pane you want to activate.

In the Text Pane, double click on a molecule folder to open and review it. Move the split bar at the bottom of the pane and open some of the subfolders. A Text Pane folder and its subfolders contain a thorough description of the molecule. Data includes molecule type and form, user-defined fields (from Vector NTI) and standard fields (such as GenBank), comments, references and so forth.

The default Analysis Pane contains three graphical representations of alignment results. Move the lower split bar and the split bars between the graphs and/or use the scroll bar to better view them.


- The first graph displays the alignment quality profile. The specific values (in a 0-1 range) are assigned to each residue at a given alignment position in each aligned

sequenced depending on whether the residue is identical, similar or weakly similar to the corresponding residue of the consensus sequence. The values for each residue at a given position are added together and the number of the sequences in the alignment normalizes the resulting value. The default values are 1, 0.5 and 0.2 for identical, similar and weakly similar residues respectively.

- The second graph displays the statistical significance profile (Absolute Complexity of an alignment. It is calculated as a sum of all pairwise residue substitution scores at a given alignment position normalized by the number of pairs in the alignment. The scores are taken from the residue substitution matrix used for alignment calculation.
- The third graph displays the statistical significance (Absolute Complexity) of an alignment for a selected molecule to the consensus sequence. The graph is calculated in a manner identical to the one in the second graph.

The Alignment Pane is described in step 4.

You can add additional analyses to the graphics pane by activating the Graphics Pane and choosing **View > List of Analyses** from the drop down menu or press the **Analysis List**

button () to open the Analysis List setup dialog box (Fig. 15.3):

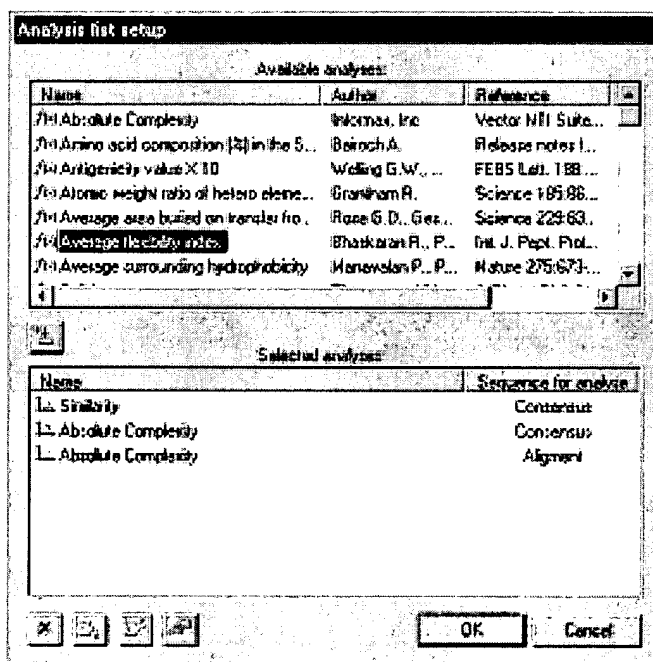




Fig. 15. 3 Analysis List setup dialog box

The dialog box lists all of the analyses available for DNA or protein, depending on the type of aligned molecules. Currently displayed analyses are listed in the Selected Analyses box. To add an analysis, select it in the Available Analyses list box and click the **Add Analysis**


button () to move it to the Selected Analyses list. Alternately, you can move an analysis to the lower list by double clicking on it.

The plot display order is determined by the order the analyses are listed in the Analysis List setup box. To modify the order, select an analysis and move it up by clicking the **Move Up**

button (). Click **OK** to close the Analysis list setup dialog box and generate the new analyses.

3. Add Molecule to Alignment

To add a molecule to your alignment, select the 41BB Human molecule in the Text Pane and

click the **Add to Alignment** button (). You can add as many molecules as you like to the alignment as long as they are listed in the Text Pane.

4. Inspect the Phylogenetic Tree

Note that now the Phylogenetic Tree Pane shows in its specific pane (Fig. 15.4).

Phylogenetic analysis is the means of studying presumed evolutionary relationships. An inferred evolutionary history is displayed in a treelike diagram suggesting the inherited relationships between the molecules. In Vector NTI, the tree is only calculated when there are more than 3 molecules in the alignment.

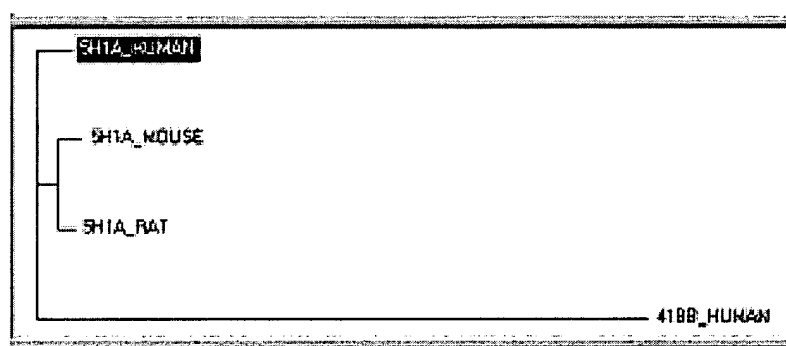



Fig. 15. 4 Phylogenetic Tree Pane

The phylogenetic tree is built using the Neighbor Joining method (NJ) of Saitou and Nei. The NJ method works on a matrix of distances between all pairs of sequence to be analyzed. These distances are related to the degree of divergence between the sequences. The phylogenetic tree is calculated after the sequences are aligned.

5. R view the Alignment Parameters and Matrix

Click the **Alignment Setup** button () on the Viewer Toolbar to see how you can adjust different parameters for the alignment. Select each of the tabs in the dialog box to quickly review the settings. Leave the last tab, Score Matrix selected.

All algorithms designed to evaluate pairwise sequence alignment are based on systems that assign scores to aligned residues, detecting similarities between differing sequences. This page displays the matrix, BLOSUM 62, used to generate your multiple alignment. Amino acids that are identical or similar in alignment score higher than those less similar. The most common of many different scoring systems are based on substitutions of amino acids in related proteins.

BLOSUM matrices are calculated from local, ungapped alignments of distantly related sequences. The matrix scores represent the minimum percent identity of the blocks used to construct the matrix; greater numbers represent lesser distances.

In Vector NTI, you can modify matrices using the Matrix Editor. Customized matrices can also be devised and stored in the database for shared or private use.

Leave the settings unchanged. It is a good idea when performing the various analyses in Vector NTI applications to run them first using the default settings. Review the results, then change the parameters as you like before running the analyses again.

6. Examine the Alignment Pane

Now move the split bars up to optimizing viewing of the Alignment Pane. Sequences of all the molecules included in the alignment are displayed here, with their names listed at the left of the corresponding sequence. Residues in the alignment are colored according to the following scheme:


<i>Alignment Color</i>	<i>Interpretation</i>
black on window default color	non-similar residues
blue on cyan	consensus residue derived from a block of similar residues at a given position
black on green	consensus residue derived from the occurrence of greater than 50% of a single residue at a given position
red on yellow	consensus residue derived from a completely conserved residue at a given position
green on window default color	residue weakly similar to consensus residue at given position

Table 15. 1 Alignment color scheme

Move the horizontal scroll bar at the bottom of the Alignment Pane. Drag the mouse across some of the sequence, selecting it. Note that the corresponding region is selected in the Analysis Pane as you do so.

The sequence names remain stationary at the left of the Alignment Pane no matter how the sequences are repositioned horizontally. If the list of molecules listed in the Alignment Pane is longer than the size of the pane, a vertical scroll bar appears as well. Vertical scrolling affects both sequences and their names but the consensus sequence remains visible at the bottom of the pane.

7. Edit the Alignment

After inspecting the alignment, press the **Edit Alignment** button () on the Viewer Toolbar to open the Alignment Editor dialog box (Fig. 15.5). This dialog box displays rows of selected molecule names and sequences.

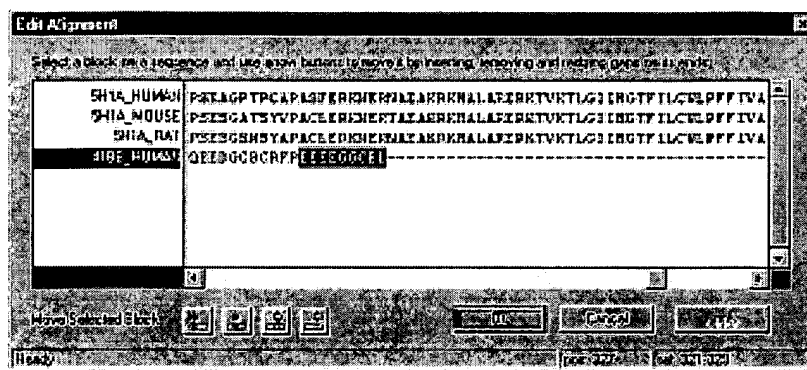





Fig. 15.5 Alignment Editor dialog box, which displays rows of selected molecule names and sequences

Scroll the sequences to find the 3' end of the 41BB Human molecule. Click and drag to select **EEEEGGCEL**. Note that as you do so, two of the Move Selected Block buttons in the

bottom of the dialog box become enabled. Click the () button, which moves the selected block one space to the right. (The () button would move the block to the extreme right of the gap.) Because editing an alignment can only occur when selected residue(s) are shifted into an adjacent gap, the arrows are only enabled when the selection block is next to a gap into which the selection may be shifted.

8. Generate Dot Matrix Plot

To perform a Dot Matrix analysis, select open a Dot Matrix window by pressing the **Dot**

Matrix button () on the main toolbar. A Dot Matrix window opens in a separate modal popup window (Fig. 15.6). In the drop-down menu at the top of the screen, select SH1A

Human (the first selected molecule in the AlignX window.) In the second drop-down menu, select, 5H1A mouse, launching the Dot Matrix.hn

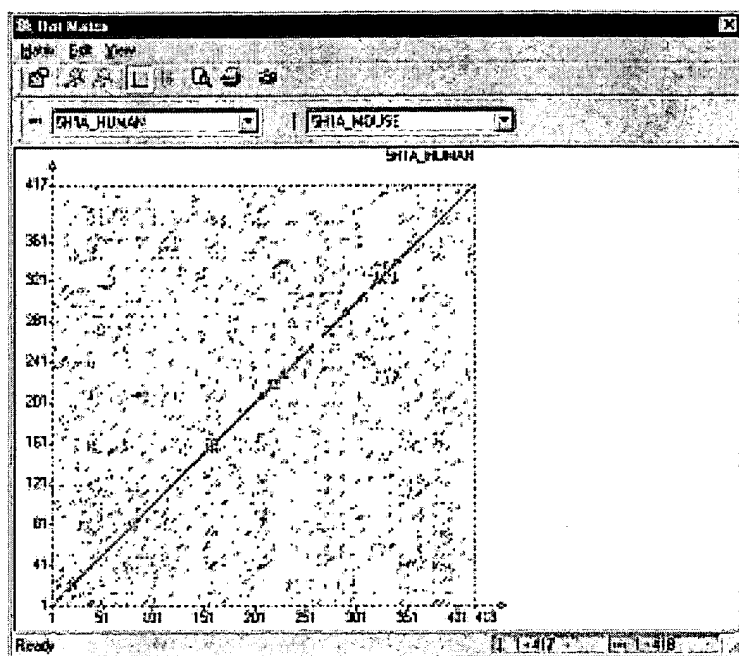


Fig. 15. 6 Dot Matrix analysis

The Dot Matrix analysis is primarily a method for finding all possible matches of residues between two sequences. One sequence (A) is listed across the top of a page and the other sequence (B) is listed down the left side.

The graphical representation of a Dot Matrix is a dashed rectangle in which any region of similar sequence is revealed by a diagonal row of dots. The molecule selected first corresponds to the horizontal axis, its name being shown in the right top corner of the Dot Matrix rectangle. The second molecule corresponds to the vertical axis. Each axis has its own scale that initially displays the sequence positions.

When the Dot Matrix is first calculated, it is shown as a representation of the full length of a molecule. Because it is difficult to analyze specific molecule regions when several thousand residues are represented, click and hold the left mouse button within the Dot Matrix rectangle while dragging the mouse, displaying the tracking dotted rectangle (Fig. 15.7).

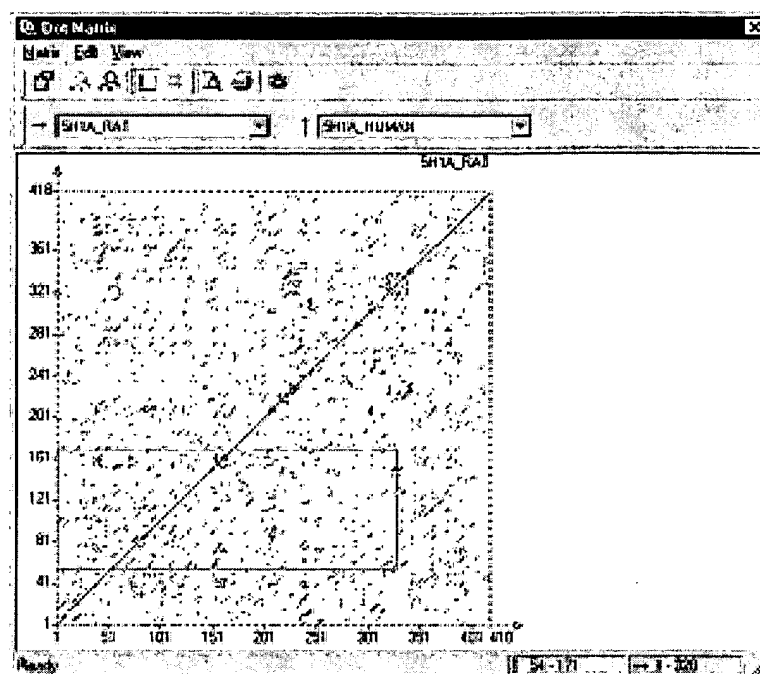


Fig. 15.7 Selected region of the Dot Matrix

When the mouse button is released, the selected region is enlarged to the entire Dot Matrix rectangle (Fig. 15.8).

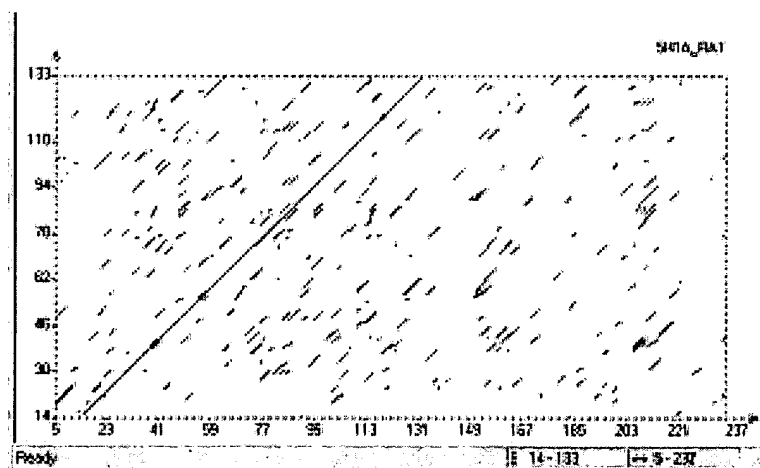



Fig. 15.8 Enlarged region of the Dot Matrix

Click the Undo Zoom () to return to the previous screen. Close the Dot Matrix window.

9. Export Alignment Molecules

AlignX is fully integrated with other components of the Vector NTI suite. As an example of exporting a molecule from AlignX into other applications, select the molecule 5H1A in the Text Pane and select **Analyze > Back Translation**. The display window that opens displays the original amino acid sequence in the upper pane, and the back translated sequence in the lower pane. In Fig. 15.9, that sequence displayed is the “most ambiguous” as shown by the scale above the sequence.

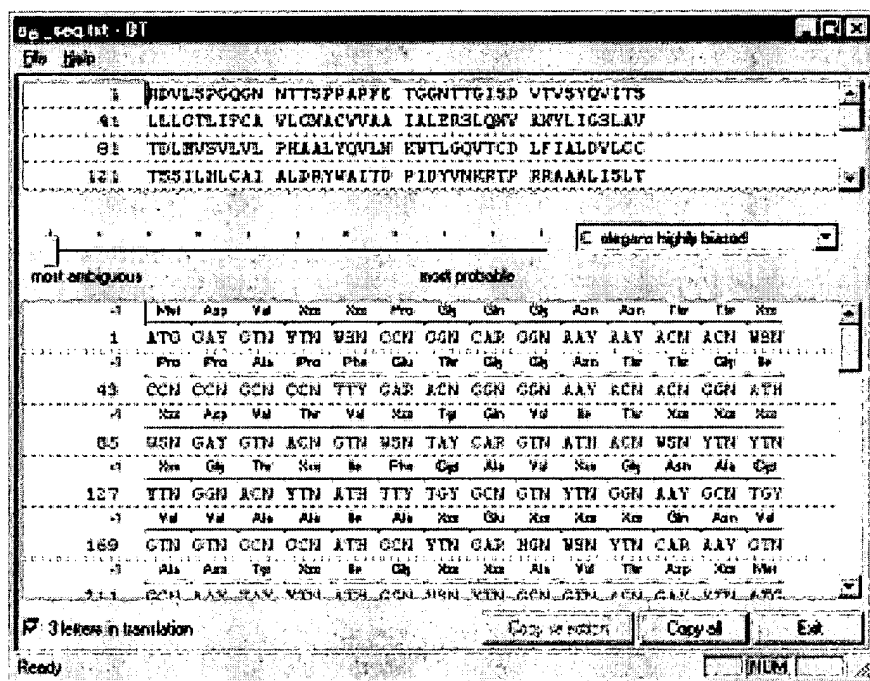




Fig. 15. 9 Back translated sequence

Drag the scale button to the right, noting how the translation code is modified as you proceed. Uncheck the “3 letters in translation” box at the bottom, changing the amino acid code to 1-letter representation. Exit the Back Translation mode.

Now select **Analyze > BioPlot – Analyze Selected Molecule**. The BioPlot display window opens, displaying physiochemical characteristics of the 5H1A Human protein. BioPlot will be discussed in the next tutorial. Close the BioPlot.

10. Copy and Print an AlignX Pane

The Copy and Print features work the same in AlignX as in the other Suite components. To copy the contents of a pane and paste them exactly as they appear in AlignX, activate the pane and click the **Camera** button (). Paste the copied material in any other application, such as a word processing document.

Print pane contents in the usual manner, by pressing the **Print** button (.

11. Save the Project and Close AlignX

You can save your project if you choose by selecting **Project > Save As** and selecting your project destination in the Save As dialog box. The next time you open Align X, the last several opened projects are listed under the Project menu option. Selecting a project from that list opens it.

Close AlignX in the same way you closed other applications of Vector NTI: select **Project > Exit**.

This ends your tutorial session with AlignX.

Chapter 17 Tutorial: ContigExpress

Introduction

ContigExpress is a program for assembling many small DNA fragments, both text sequences and chromatograms from automated sequencers, into longer contiguous sequences or “contigs”.

Work in ContigExpress takes place in a ContigExpress Project, a file where you store fragments, their assemblies, and assembly options related to your current task. In ContigExpress, fragments can be edited directly, with the chromatograms in full view. Changes are tracked and a history is maintained. The contigs generated can then be saved, exported into other applications of the Vector NTI Suite or exported to third-party tools available on the WWW.

This tutorial can be completed in one session or divided into three sessions as noted. At the end of this tutorial, you will be able to:

- Create a ContigExpress project
- Navigate and manipulate the CE Project Explorer window
- Assemble contigs
- Edit fragments and contigs in the Fragment Window or the Contig Window

Follow the steps of the tutorial in the order shown. Figures show what your screen should look like at various points along the way.

Session 1 Project Explorer

1. Open a ContigExpress Window

Launch ContigExpress (CE) by selecting it in the program group or folder in which you installed the Vector NTI Suite. *You can also launch ContigExpress from most other Vector NTI Suite applications.* ContigExpress opens with an empty Project Explorer.

2. Open a CE Project and Add a Fragment to the Project

To open the project, in the Project Explorer window select **Project > Open Project**. Browse for the Vector NTI Suite\Demo Projects folder (this is found under Program Files if a default installation has been performed). Open the Demo Projects folder and double-click on Demo Projects.cep to open the project in the Project Explorer window. There will be 13 fragments listed in the List Pane of the Project Explorer (Fig. 17.1):

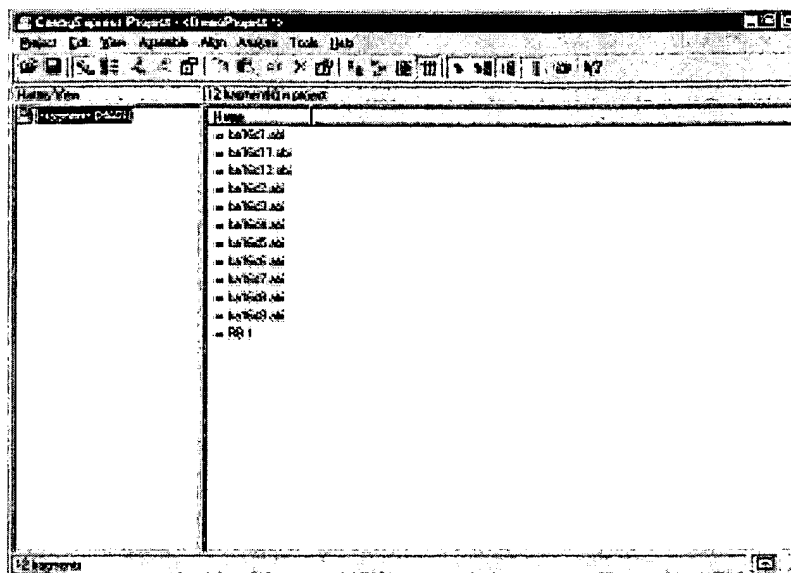


Fig. 17. 1 Fragments listed in the List Pane of the Project Explorer

Most files saved from an automatic sequencer have an .abi extension. Add one other fragment to the list by selecting **Project > Add Fragments > From ABI file...**

This option opens the Import Sequence From dialog box (Fig. 17.2). Select Sample1303.abi and click **Open**.

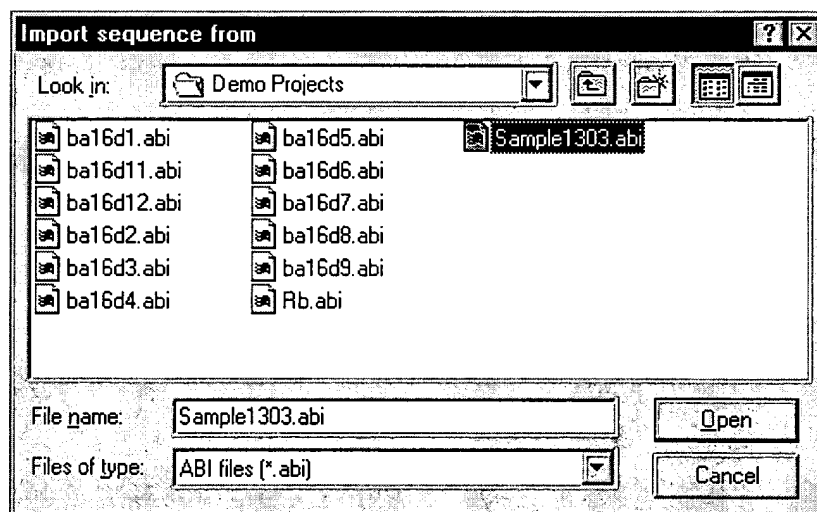


Fig. 17. 2 Import Sequence From dialog box

Note: Sometimes the names of the imported fragments do not directly correspond to their original file names – ContigExpress assigns names that are found inside the imported files to the newly imported fragments. (In this example, *Sample1303.abi* becomes *xb-control* on the list).

3. Examine the CE Project Explorer Window

As you noted, CE opens to an empty Project Explorer window to which you added a list of molecules. The window is similar to other Vector NTI Suite windows with a title bar, menu bar and tool bar (Fig. 17.3). The Tree Pane on the left and the List Pane on the right are divided by a movable split bar. A Status Bar at the screen bottom displays the Project Explorer status.

All of the toolbar buttons are summarized in Chapter 4. Toolbar buttons are described in this tutorial as needed.

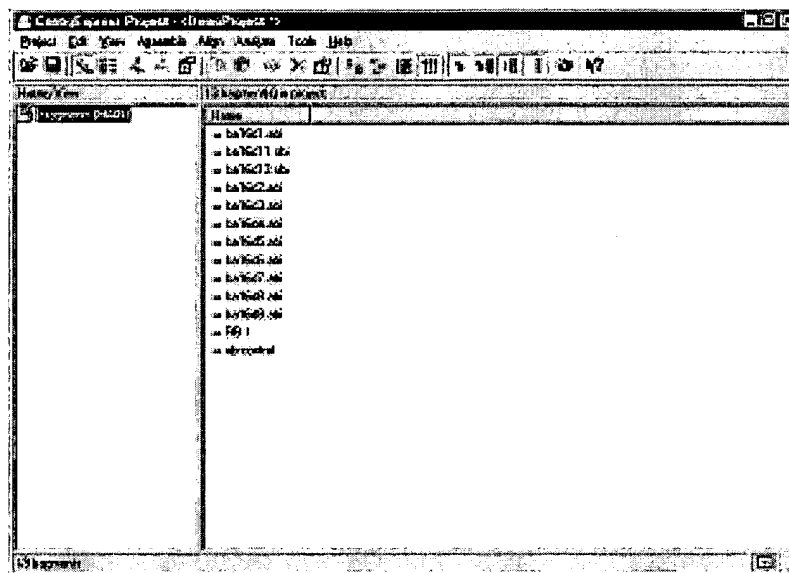


Fig. 17. 3 Examining the CE Project Explorer window

4. Create Assemblies of Contigs

Choose the Assembly Algorithm

There are two different assembly algorithms to choose from in CE, Linear Assembly and Pairwise Assembly. The advantages and disadvantages of each algorithm are discussed in Chapter 29 (page 469). For the purposes of this tutorial, we will use the Pairwise Assembly algorithm.

From the menu, choose **Assemble > Assembly Setup** or choose the **Assembly Setup** button on the toolbar. The Assembly Setup dialog box (Fig. 17.4) appears (more detailed information about Assembly Setup can be found in Chapter 29):

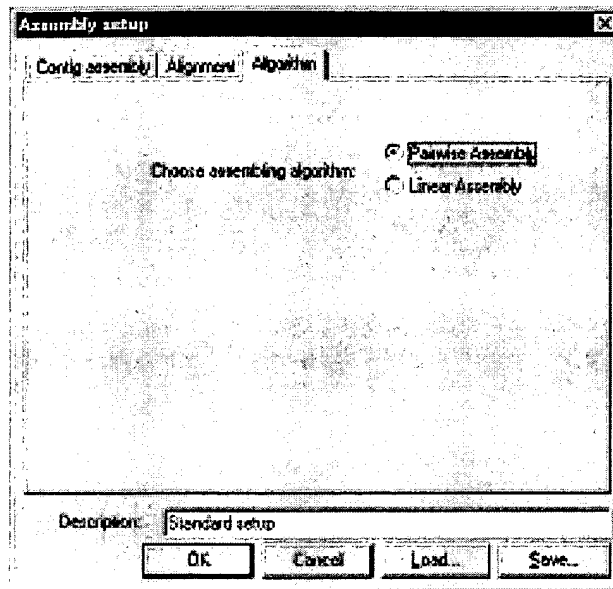



Fig. 17. 4 Assembly Setup dialog box

Choose the **Algorithm** tab on the Assembly Setup dialog box. Select the Pairwise Assembly radio button and press the **OK** button to register the change and close the dialog box.

Assemble Sequences

To assemble a contig, select all fragments except xb-control, with **SHIFT + CLICK** at the names beginning and ending the list at RB 1. Click the **Assemble Selected** button (). *This button is enabled only when at least two fragments are selected.*

(If you want to select all the sequences in the CE Project window for assembly, right click on the first sequence in the list and choose Select All from the shortcut menu.)

An Assembly Progress dialog box allows you to monitor the assembly process or cancel the assembly at any time.

After the contig assembly is completed, the Tree Pane shows an assembly (Assembly 1). Select the assembly with a click. All of the project fragments are listed in the List Pane, beginning with the contig (Contig 1) made from two fragments (Fig. 17.5). An assembly can be made up of one or more contigs.

[illegible]

5. Review and Modify Viewing Options

Options

Details View | Status Bar | Copy Fragment/Config

Available Fields:

- 3' Trimmed bases
- 5' Trimmed bases
- Comment
- Date created
- Date modified
- Description
- Original length
- Status
- Type

Displayed Fields:

- Name
- Length

Order:

Move Up

Move Down

Alignment:

☒ Left

☐ Right

☐ Center

OK Cancel Apply Help

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This dialog box, similar to those in other Vector NTI Suite applications, has three tabs. The **Details View** tab controls the information displayed in the List Pane of Project Explorer. The **Status Bar** tab controls the information displayed on the Status Bar for a selected item in the CE project explorer. The **Copy Fragment/Contig** tab controls the copy format for fragments and contigs.

On the **Details View** tab, the top to bottom order of the Displayed Fields corresponds to the left to right order of columns in the List Pane. In the Displayed Fields pane, select Length line, and click the **Move Up** button. Click **OK**. Note that Length now is the column to the immediate right of the fragment names in the List Pane.

On the toolbar, the List Format group of buttons control the list display (Fig. 17.9):



Fig. 17. 9 List Format group of buttons

Click each of these buttons and observe how the information display of the Project Explorer changes. Leave the **Details** button () selected.

The next group of buttons allows you to filter data displayed in the List Pane (Fig. 17.10):

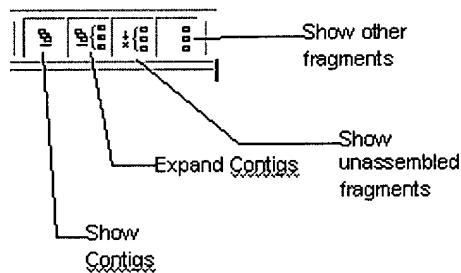







Fig. 17. 10 Filter Data group of buttons

By default all four of these buttons, or viewing filters, are selected, showing the maximum amount of information. To see how they work, make sure that Assembly 1.1 in the Tree Pane is selected. Note that no matter how you display the list, all fragments are still identified by one of the following icons:

- Contigs ()
- Fragments included in the assembly ()
- Fragment not included in the assembly ()

- Fragments not considered for the assembly ()

Now click the **Show Contigs** button (), turning it “off”. Contig 2 and its fragments, ba16d8.abi and ba16d9.abi are no longer displayed in the List Pane (Fig. 17.11):

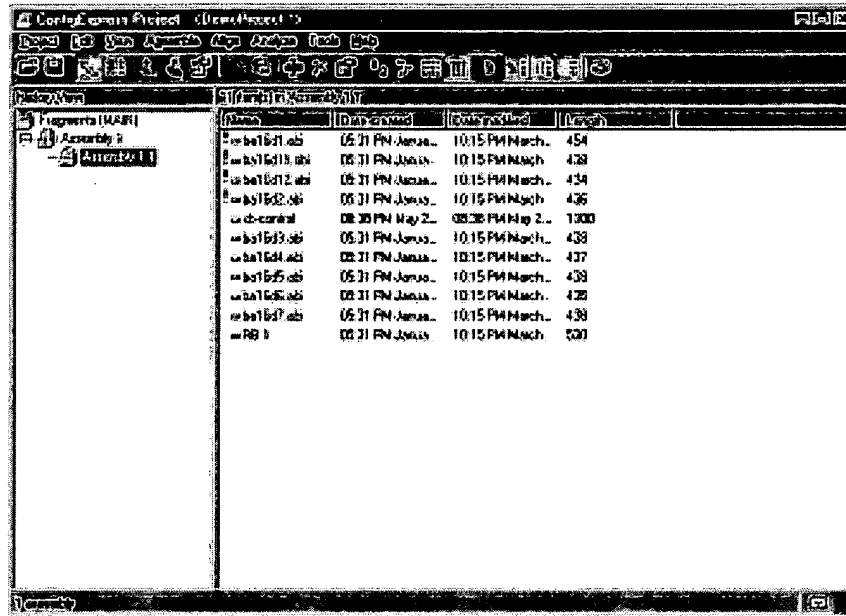





Fig. 17. 11 Using the Show Contigs button

Click this button again to restore the original display, then click **Expand Contigs** button (), turning it “off”. Now the fragments included in Contig 2 are excluded from the display (the contig is not “expanded”):

Click the Expand Contigs buttons again to restore the original display. Click each of the remaining two buttons, turning them off then on again in turn: the **Show Unassembled Fragments** button () and the **Show Other Fragments** button (). Note the fragments that are excluded and included in each display. Finish with all filters enabled (all buttons pushed) as it was in the original display.

You can use any combination of enabled and disabled data filter for list display.

6. View Contents in the Tree and Content Panes

The currently enabled viewing mode in the Tree Pane is called the History View. It shows the historical relationship between the assemblies in a tree form.

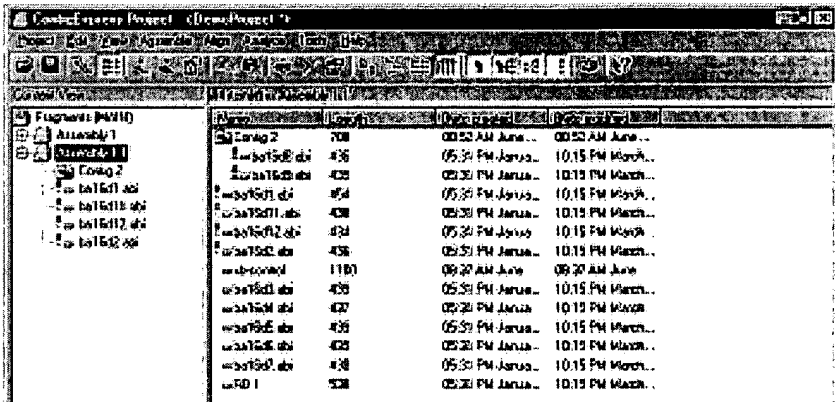


Fig. 17. 12 Viewing contents in the Tree and Content Panes



7. Manage Fragment Lists and Project Items

ContigExpress can use the Windows clipboard for copy and paste operations.



(ae)

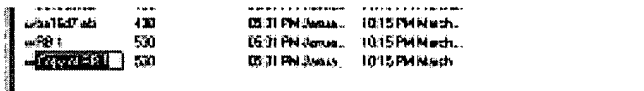


Fig. 17. 13 Renaming project items

In the text box, change the name to **123** and press **Enter**, renaming the fragment.

Select **123** and click the **Delete from Project** button (X). Confirm the action. Fragment 123 is deleted from the project and the display is reset to the upper level of the project (Fragments (MAIN)).

Select **Assembly 1.1** and **fragment ba16d8.abi** in this assembly. Click the **Delete from Project** (X) button. ContigExpress warns you that the selected fragment is included in several assemblies and deleting this fragment will delete them as well (Fig. 17.14).

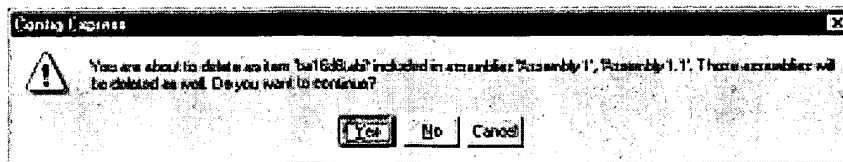


Fig. 17. 14 Deleting items from a project

Click **No**, indicating the deletion will not continue. If you had clicked **Yes**, all assemblies would have been deleted.

8. Save and Close a CE Project

Even though you may wish to continue the tutorial, save and close the project and then open it again to become familiar with these functions.

To save the project, select **Project > Save As**. Name the project **Tutorial Project** and select the Demo Projects as the destination.

To close your project, select **Project > Close Project**. ContigExpress reverts to its initial state, with an empty workspace. You can either start a new project by importing the fragments or open an existing project from the disk.

Exit ContigExpress as you would close any other Windows application.

Session 2 Working in Fragment Window

1. Open and Inspect the Fragment Window

Open the CE Project Explorer and select Project on the menu bar. The name of the project you worked with last is shown in the used projects list at the bottom of the drop down menu. Select Tutorial Project, opening it in Project Explorer. In the Project Explorer List Pane, double-click the fragment **ba16d12.abi** to open it in a Fragment window. Maximize the window and arrange the panes conveniently by dragging the split bars with a mouse.

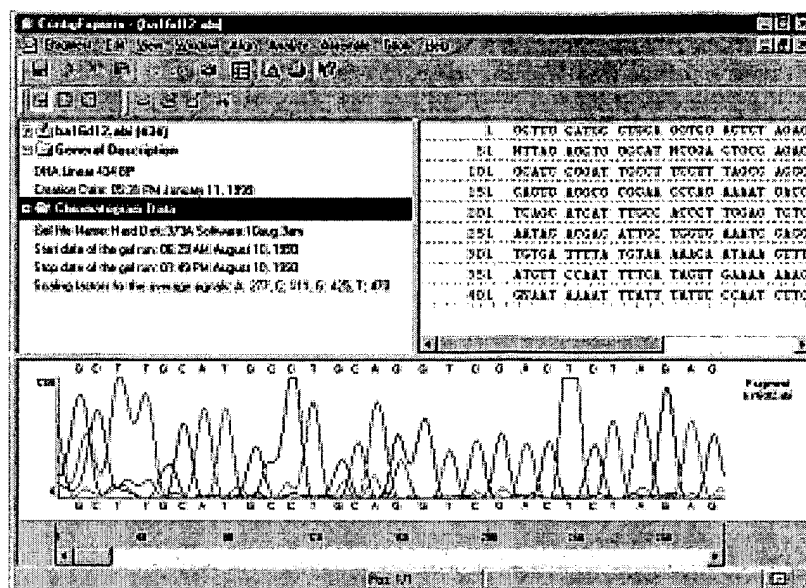



Fig. 17.15 Fragment Display window

The Fragment Display window consists of a title bar, a menu bar, three toolbars and three panes: a Text Pane, a Sequence Pane and a Chromatogram Pane (Fig. 17.5). The panes are divided by movable split bars.



All of the toolbar functions are summarized in Chapter 4. Toolbar buttons are described here as needed.

Text Pane

Click the **Text Pane** button () to activate it. The Text Pane lists properties of the fragments, such as its name, general description, chromatogram data (if any), features.

Click the + to the left of the General Description line to open and view the folder contents. Double click on the folder to close it.

Sequence pane

Click the **Sequence Pane** button () to activate it. Only one strand of the sequence is shown by default. Click the **Show Two Strands** button () , now displaying both strands of the sequence (Fig. 17.16):

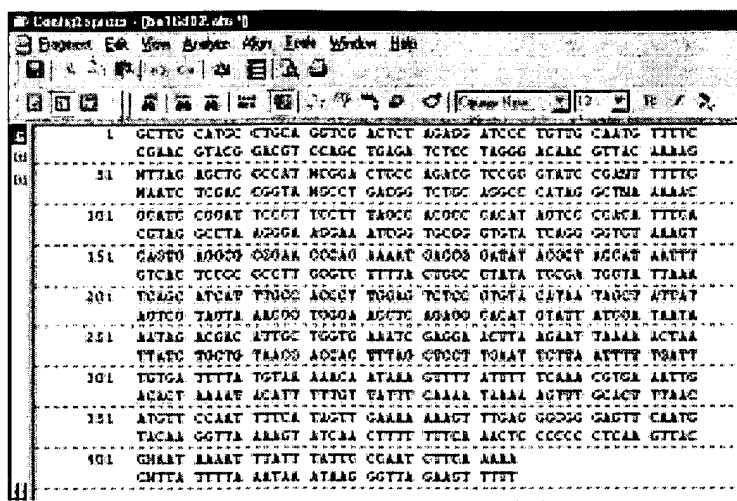



Fig. 17. 16 Displaying two strands of a sequence

To modify the number of sequence blocks displayed per line, clicking the **Lane Width**

button () and enter **15** in the Blocks per Line: field of the dialog box. Click **OK** and the number of blocks per line increases as directed (Fig. 17.17).

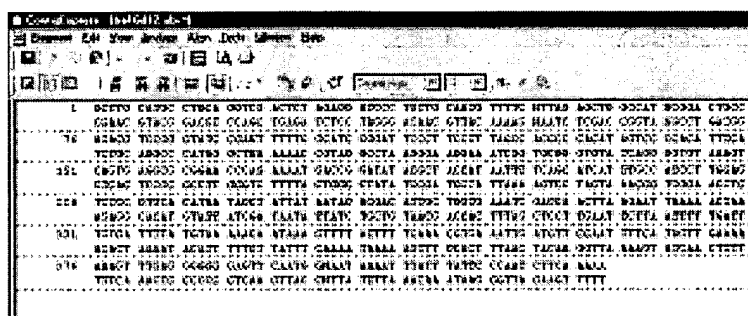


Fig. 17. 17 Modify the number of sequence blocks displayed per line

Using the same technique, change the blocks amount back to 10.

Chromatogram Pane

Click the **Chromatogram Pane** button to activate it (Fig. 17.18). The vertical scroll lever on the left side of the Chromatogram Pane can be used to resize chromatogram peak height.

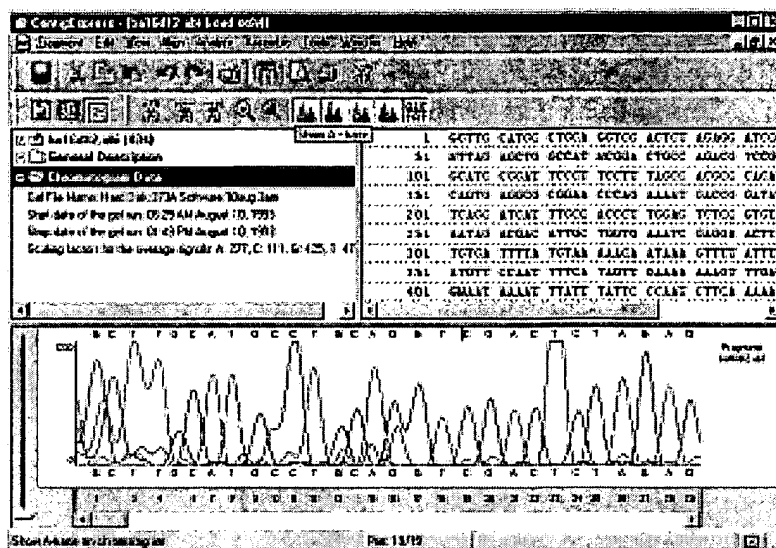




Fig. 17.18 Chromatogram Pane

Individual base traces can be displayed or removed by pressing the corresponding **Show**

Trace toggle buttons () on the toolbar or by choosing **View > Show Traces** for the particular base you want to show or hide. For example, if you want to hide the chromatogram trace for Ts only, make sure that the **Show Trace - T** button is not pressed, but that the **Show Trace - A, C and G** buttons are pressed. Press each button to see the effect on the Chromatogram Pane.

2. Sequence Selection Techniques

In ContigExpress, the cursor in the Sequence Pane becomes a short line vertical blinking I-beam called a *caret* () . A selected sequence is highlighted and marked at one end (the "active" end) with a caret. The current position of the caret is always shown in the Position Box on the status bar (Fig. 17.19):

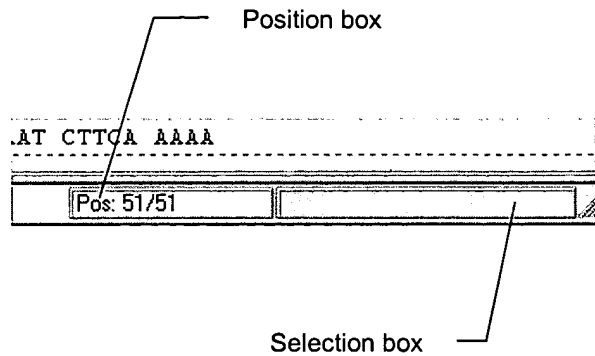


Fig. 17. 19 Position Box on the status bar

Click this box to open a Set Caret Position dialog box (Fig. 17.20) that can be used to position the caret anywhere in the sequence:

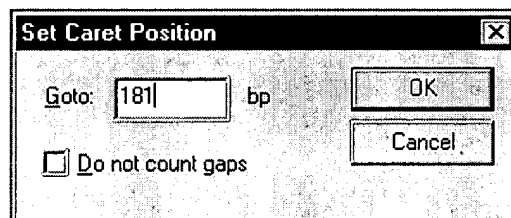


Fig. 17. 20 Set Caret Position dialog box

Click on the Selection box on the Status Bar, opening the Set Selection dialog box (Fig. 17.21). Set the selection to 10 bp – 30 bp and press **OK**:

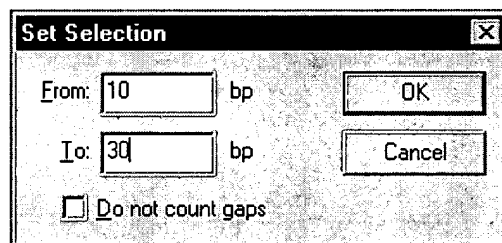


Fig. 17. 21 the Set Selection dialog box

The selection is highlighted in the Sequence Pane and shown in the Selection Box on the status bar (Fig. 17.22):

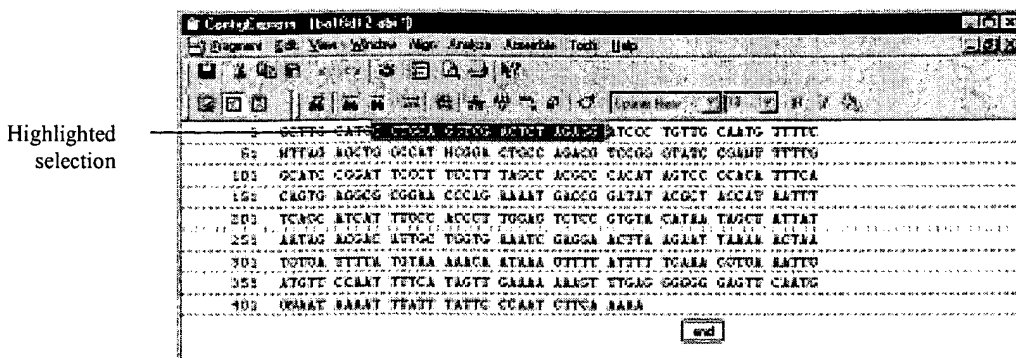


Fig. 17.22 Highlighted selection in the Sequence Pane

To modify the 5' and 3' ends with the mouse:


- Click the 5' end of the selection, moving the caret [note its change to a 5' mark (Φ)] to that position and activating the 5' end. **CLICK + DRAG** the 5' end to a position near the start of the molecule. *Observe the selection box as you drag.* The caret remains with the end of selection you are dragging.
- The end of the selection marked with the caret can also be moved with arrow keys on the keyboard. Hold the **SHIFT + CLICK** the left or right arrow keys to move the 5' end of selection one nucleotide at a time. (If you hold down **CTRL + SHIFT +** use the arrow keys, the selection is changed 5 nucleotides at a time.) When the 5' end is placed on the start of the molecule (1 bp), release the **SHIFT** key.
- To set a selection with the mouse, click anywhere in the blank area inside the sequence and drag the caret with the mouse, releasing when the selection is complete.

Note that the selected fragment is also displayed concurrently in the Chromatogram Pane. This occurs no matter where the selection was made.

3. Display ORFs and Translate the Nucleotide Sequence

Display ORFs for the Sequence

In the Fragment Window, activate the Sequence Pane and change to double-stranded sequence display using previously described techniques. Choose **View > ORFs** from the

menu or press the **Show ORFs** button () on the toolbar. ORFs are displayed for the direct and complementary strands (Fig. 17.23):

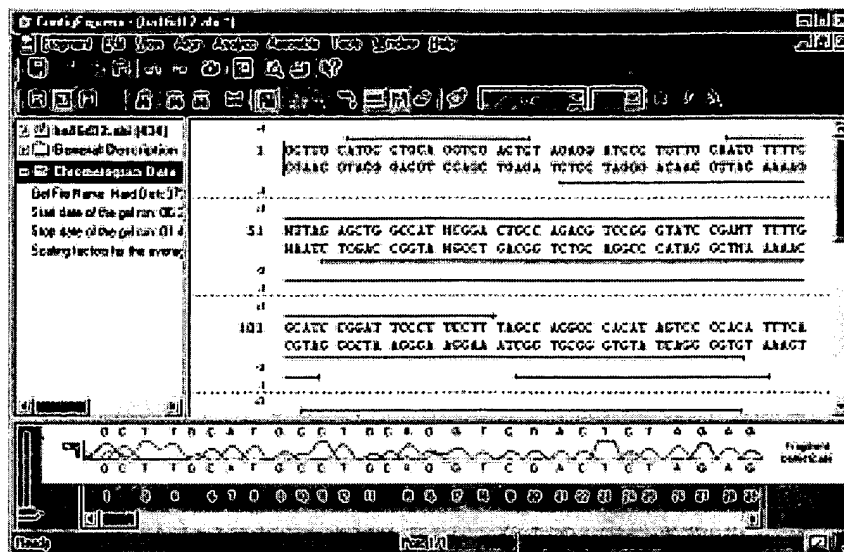



Fig. 17. 23 Displaying ORFs

If single-stranded sequence is displayed, only the ORFs for that strand are shown.

Translate the Nucleotide Sequence

Select the ORF region from 7 to 24 bp. The Chromatogram Pane has also made the corresponding selection and has scrolled its graph to make selection.

Now click the **Translate Selection** button () on the Window Toolbar. ContigExpress shows the amino acid abbreviations above the selected sequence (Fig. 17.24):

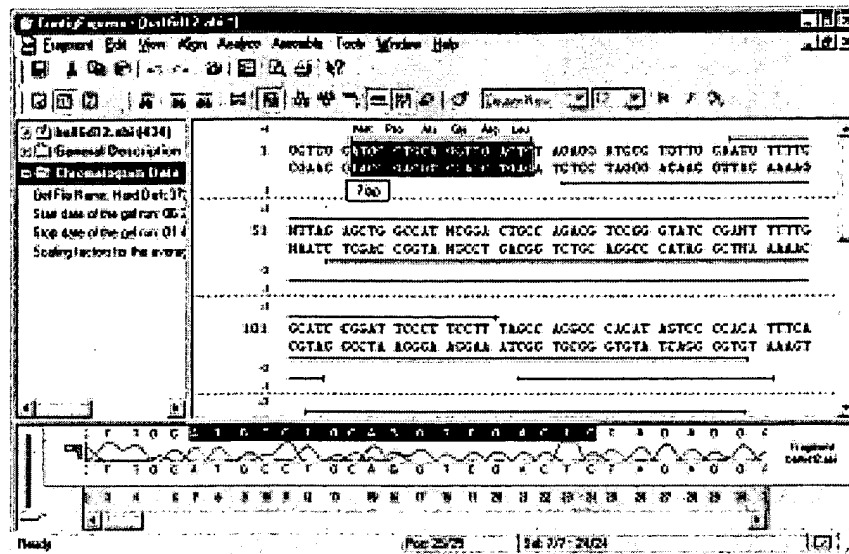



Fig. 17. 24 ContigExpress shows the amino acid abbreviations above the selected sequence

To translate the complementary strand of the selection, click **Translate Complementary** button (). If the sequence is displayed as single-stranded, the pane automatically switches to two-strand mode to show the complementary translation below the selection (Fig. 17.25):

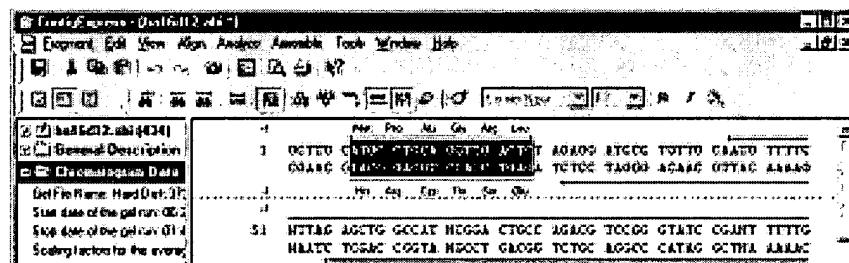






Fig. 17. 25 Translating the complementary strand of the selection

Click the **Show 1-letter AA codes** button () to cycle between 1 and 3 letter abbreviations in the translations.

To delete all translations, click **Clear All Translation** button () [an “eraser”]. Also click **Show Two Strands** button () to return to 1-strand display.

Notice when you return to single-strand sequence display, the ORFs display changes to direct ORFs only. Click on the **Show ORFs** button () to remove the ORFs display.

4. Edit the Nucleotide Sequence in the Sequence Pane

To edit in the Sequence Pane, make a selection from 176 to 180 bp. Press the **DELETE** key on your keyboard. The selected symbols are moved under the line, with downward pointing arrows (↓) taking their place (Fig. 17.26):

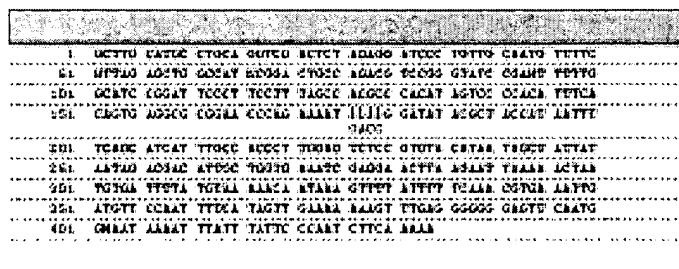


Fig. 17. 26 Editing the nucleotide sequence in the Sequence Pane

The arrows in place of the deleted symbols show where residues have been deleted. The residues below the arrows show what has been deleted.

Now move your attention to the Chromatogram Pane. There is a sequence above and one below the graph. Before we started editing, they were identical. The sequence above the graph reflects all your editing commands, the sequence below always remains in its original form, thus giving you a useful reference to track and compare your changes. Note that the deletion resulted in each deleted nucleotide being replaced by a dash (-) in this sequence.

Back in the Sequence Pane, move the caret in the Sequence Pane to position 171 and type "TTT". Three symbols with upward pointing arrows (↑) below them are inserted at the caret position (Fig. 17.27):

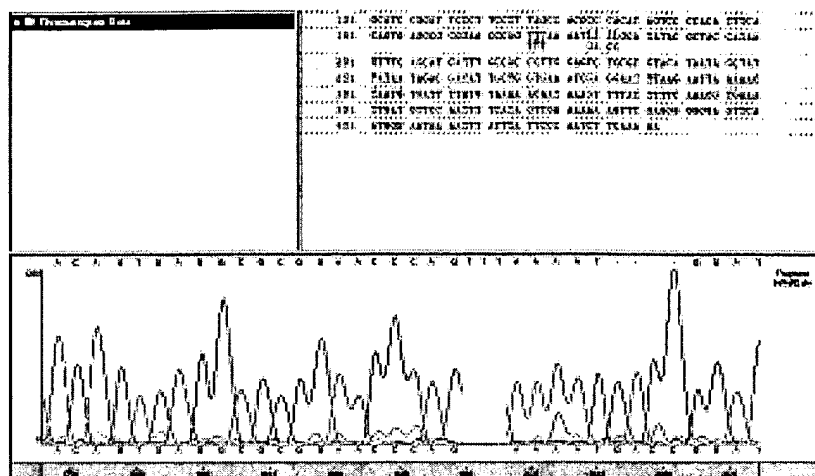


Fig. 17.27 Arrows (\uparrow) are inserted at the caret position in the Sequence Pane

In this case, the arrows indicate that symbols were inserted in a sequence (“added to” the sequence) into a position, not substituted for other nucleotides. The sequence above the graph in the Chromatogram Pane also shows the insertion (in another color). Note that a gap appeared in the graph because there is no data to build chromatograms for newly inserted symbols.

Make a selection from 175 to 176 bp and type “GG”. The symbols “AA” that were selected are replaced by “GG”, and “AA” is shown below “GG” to help you track the changes (Fig. 17.28):

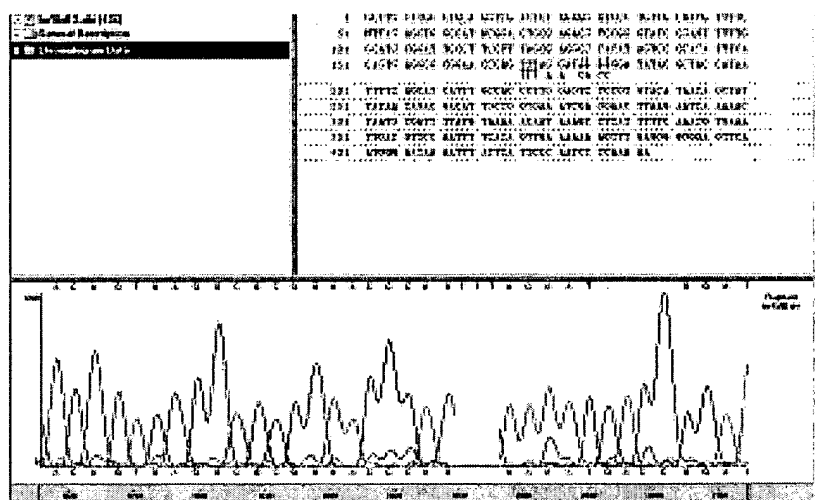



Fig. 17.28 Tracking changes in the Sequence Pane

In this case, the chromatogram does not change because the “GG” symbols were not inserted; they just replaced exactly the same number of original residues.

By default, the peaks for deleted bases are still shown in the chromatogram. This means that although bases have been deleted, they are still considered part of the sequence during translation. Deleted peaks can be removed using the Show Deleted Peaks toggle function, allowing translation in the new/resulting reading frame.

Press the **Show Deleted Peaks** toggle () button or choose **View > Show Deleted Peaks** from the menu to turn off the Show Deleted Peaks function. When a message appears indicating the fragment will switch to read only mode, choose **Yes**. Make a selection across the area where you have deleted bases from 166 to 186/182 bp and translate the sequence using techniques previously described. Notice the translation considers the deletions in the sequence and proceeds according to the new frame (Fig. 17.29).

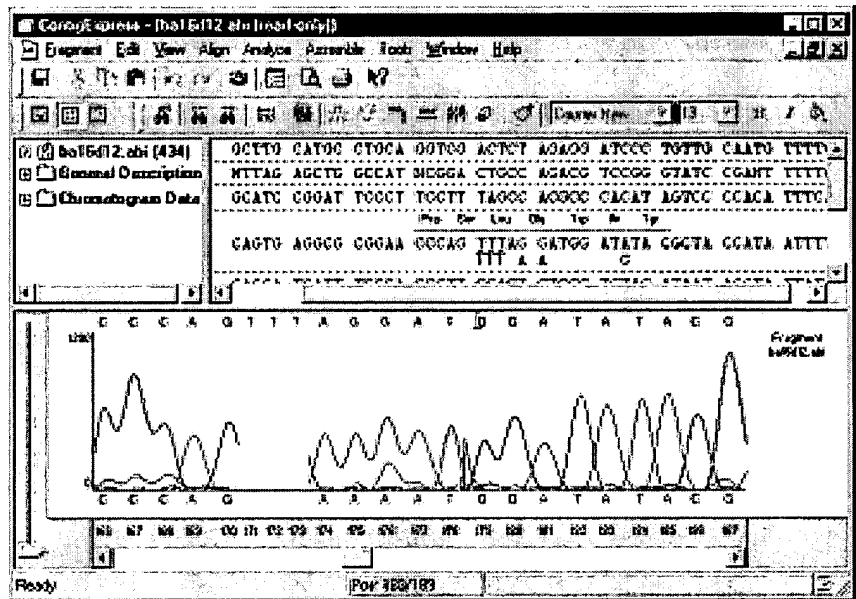


Fig. 17. 29 The translation considers the deletions in the sequence and proceeds according to the new frame

Press the **Show Deleted Peaks** button () again to reactivate the function. Notice the translation now includes the peaks, as if no deletions have been made (Fig. 17.30).

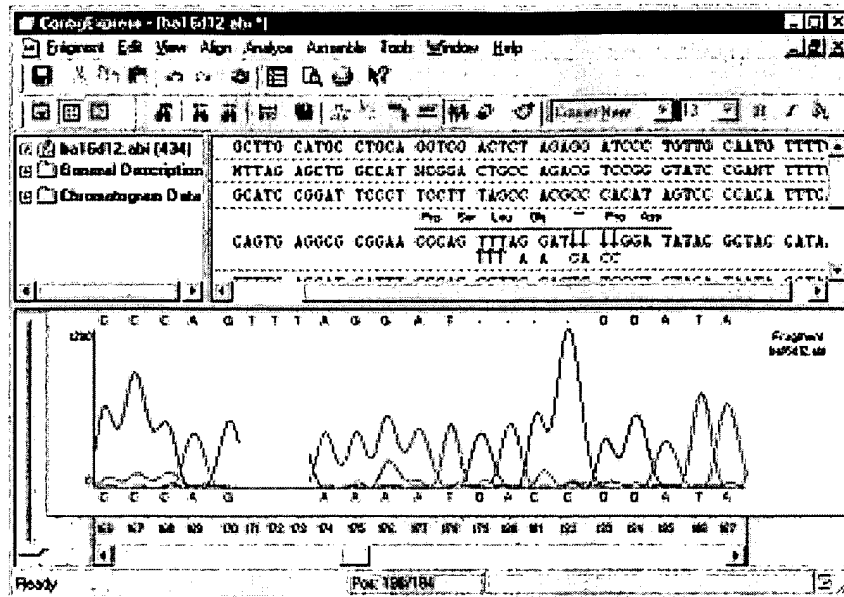


Fig. 17. 30 Using the Show Deleted Peaks button

Erase your translation using techniques described earlier.

You can use the Undo and Redo commands to reverse or restore your editing actions. If you click the **Undo** (↶) Main toolbar button several times, it becomes disabled and both Sequence and Chromatogram panes return to their original sequences.

5. Edit a Sequence in the Chromatogram Pane

Activate the Chromatogram Pane active by clicking the Chromatogram Pane (📊) Window Toolbar button.

In the top sequence in the Chromatogram Pane, select "T" at bp 175 and type **C**. As in the Sequence Pane, "T" is replaced by "C". Note that each of these actions is simultaneously reflected in the Sequence Pane.

Now select "GG" at bp 180 – 181 and type **TTT**. Notice the differences each time you enter **T**. The first two are considered to be replacement residues (since you have selected 2 symbols before typing). The third one is treated as an insertion because all selected symbols are already replaced.

Play with editing in both panes to become familiar with all features, but do not be afraid to edit too much. You can restore the original sequence by selecting **Fragment > Revert to Saved**.

Note that all editing operations are reflected in both the Sequence and Chromatogram Panes. They can be summarized as follows (NTs = nucleotides):

<i>Action</i>	<i>How to Perform</i>	<i>Sequence Pan Result</i>	<i>Chromatogram Pane Result</i>
Delete	Select residues; press Delete	(↓) replaces NTs; NTs moved below strand	(- - -) appear in upper sequence
Insert	Place caret; type new NTs	(↑) appears below new NTs; new NTs are colored	A break appears in the chromatogram
Replace	Select NTs; type new NTs	New NTs appear in strand; replaced NTs moved below strand	New NTs appear in upper sequence; no break in chromatogram

Table 17. 1 Editing operations for Sequence and Chromatogram Panes

6. Close the Fragment Window

Select **Project > Close** to close the Fragment window. If asked, confirm the operation.

7. Edit and Save a Fragment Included in an Assembly

Return to ContigExpress Project Explorer. In the Tree Pane, double click on Assembly 1, opening it in the List Pane. Double-click fragment **ba16d8.abi** in Contig 1.

In the Fragment window, go to any position in the Sequence pane and type a **T**.

Because this fragment is included in at least one assembly; changing the length of an assembly fragment renders the assembly to be invalid. Therefore, the assembly would be deleted if this edit fragment proceeds. You are warned about that (Fig. 17.31):

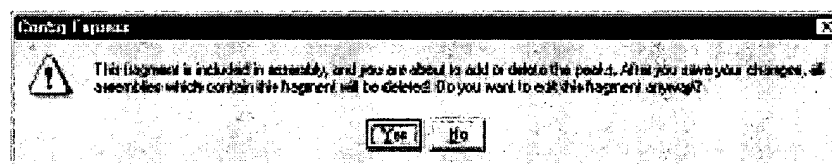



Fig. 17. 31 Warning regarding deleting the edit fragment s

Click **Y**es to continue editing. **T** is inserted in the sequence.

Close the Fragment Window by clicking the **Exit** button (). You are again warned that saving the fragment with a changed length will delete all assemblies containing this fragment.

Select **Yes**. The Fragment Window closes and you are returned to Project Explorer. Note that all assemblies have disappeared because they all contained the fragment we have just edited.

Note: If editing a fragment doesn't change its total length (for example, you have replaced one symbol with another), then the assemblies containing it are not discarded.

8. Exit ContigExpress

Close ContigExpress by selecting **Project > Close Project**. When asked whether to save the changes, click **No** so you can continue to use the project with the assemblies created in the first part of this tutorial.

Session 3 Working in the Contig Window

Selecting and opening a fragment brings up Fragment Window. Selecting and opening a contig, opens a Contig Window.

1. Open and Examine a Contig Window

Re-open ContigExpress as you have done before. Load the Tutorial Project that you created in Session 1 by selecting **Project > Tutorial Project**. In the Project Explorer window, select Assembly 1 and double-click on Contig 1 in the List Pane, opening the Contig Window (Fig. 17.32). Maximize the window.

Contig Window has many elements similar to the Fragment Window: a title bar, menu bar and two tool bars and three panes: a Text Pane, a Graphics Pane and an Alignment Pane. Arrange the panes conveniently by dragging the split bars with a mouse. A Status Bar at the screen bottom displays the Project Explorer status.

All of the Contig Window toolbar buttons are summarized in Chapter 4. Toolbar buttons are described in this tutorial as needed.

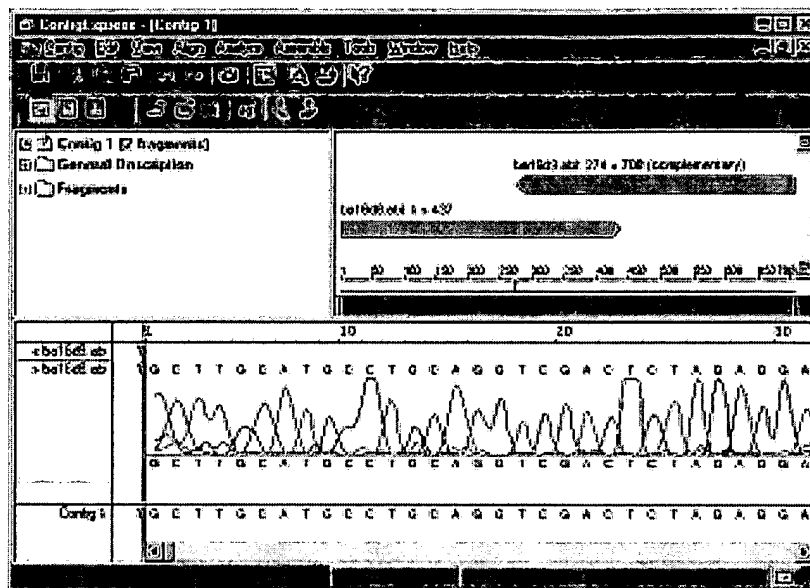




Fig. 17. 32 Contig Window

Text Pane

To activate the Text Pane, click on the **Text Pane** button (). The Text Pane is similar to other Text Panes with its folders containing various properties of the fragment.

Open the Fragment Folder and select fragment **ba16d9.abi**. Choose **Edit > Find** or click the **Find** () button. This fragment is located in the remaining two panes: in the Graphics pane it is highlighted in blue and in the Alignment pane its name is highlighted (Fig. 17.33):

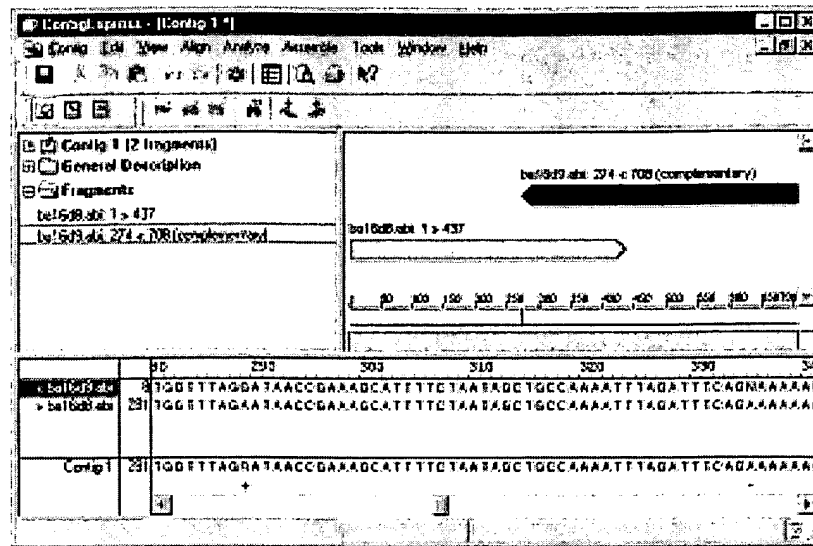




Fig. 17.33 Using the Text Pane on the Contig Window

It is possible to perform additional assemblies from the Contig Window without returning to the CE Project Window. The **Assemble Selected** toolbar button () becomes accessible when two or more sequences are selected in the Text Pane. The **Assemble All But Selected** () button becomes available when there are at least two unselected sequences and one selected sequence in the Text Pane (Fig. 17.34). The Assemble All But Selected feature is similar to Assemble Selected, except that it excludes the selected fragment(s) when attempting to assemble a Contig.

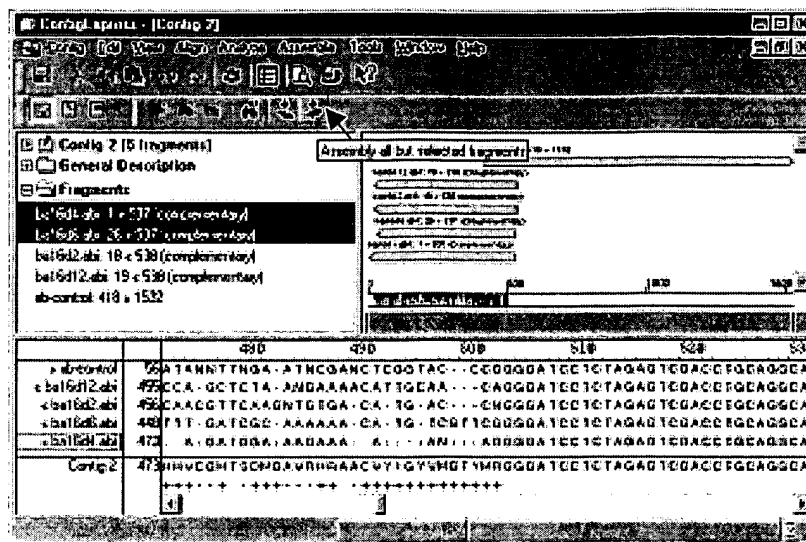



Fig. 17. 34 Assemble All But Selected feature excludes the selected fragment(s) when attempting to assemble a Contig

When either of these commands is executed, the new assembly is performed and added to the CE project as a child assembly of the assembly that the current Contig is a part of. For example, if the Contig is part of Assembly 1, the new assembly will be called Assembly 1.1.


Graphics Pane

Click the **Contig Graph** button () in the Window toolbar to activate the Graphics Pane.

The Graphics Pane contains horizontal arrows representing the relative positions of the fragments forming the contig. The arrowheads indicate the direct or complementary position of the respective fragment.

Below the fragment arrows is a scale, or Weight Graph, with position markers for this contig. The Weight Graph displays the quality of the assembly. Move the scroll bar on the Weight Graph so that you can view the region at about position 270. Note the green vertical line there. Position the cursor at that site and click.

By default, the data in this pane is drawn in "Fit to screen" mode.

Click the **Zoom In** button () many times to enlarge the graph. You may need to use the horizontal scroll bar to keep the site you marked in view as you enlarge the area. *When the maximal zoom is reached, the Zoom button becomes disabled.*

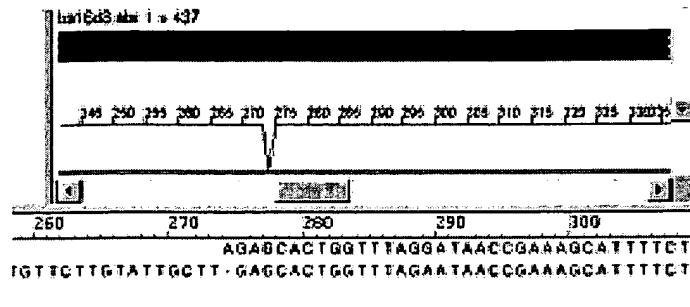


Fig. 17. 35 The nucleotide at bp 274 appears to be unresolved

The negative peak in the Weight Graph line indicates a position where the assembly is poor. In the Alignment Pane, look at the symbols at the same position. The nucleotide at bp 274 appears to be unresolved (Fig. 17.35). Instead of having to thoroughly examine all nucleotides sequences throughout the assembly, you can see the “bad” points with just one glance at the Weight Graph.

For details regarding the weight calculation, refer to Chapter 30.

Click the **Fit to Screen** toolbar button (🔍) to return to the standard display mode.

You can make selections in the Contig Window frames using techniques you have already learned. Select nucleotides from 435 to 483 bp. Note that the selection in the Contig Graphics Pane is concurrently selected in the active fragment of the Alignment pane, scrolled to show this selection (Fig. 17.36):

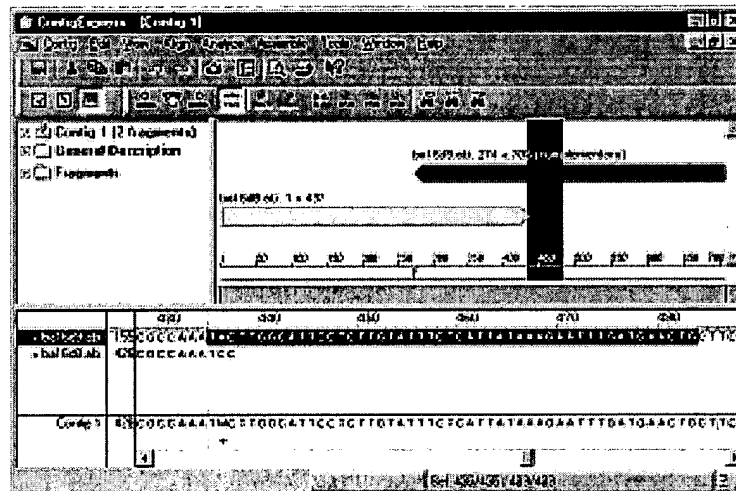



Fig. 17. 36 Selecting nucleotides 435 to 483 bp

ORFs can be displayed in the Graphics Pane of the Contig Window by activating the Graphics Pane and either choosing **View > Show ORFs** from the menu or pressing the **Show ORFs** button () on the toolbar. Click the **Show ORFs** button.

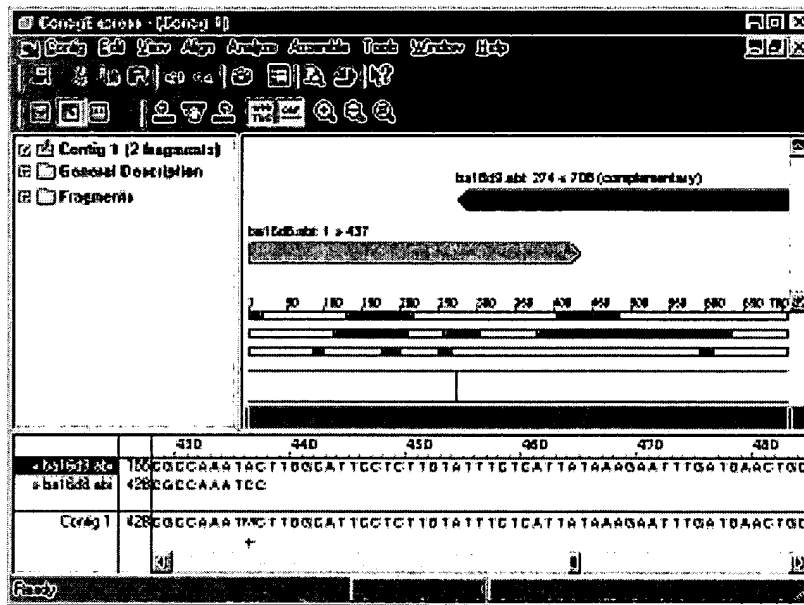


Fig. 17. 37 The ORFs display area appears below the fragment display in the Graphics pane

The ORFs display area appears below the fragment display in the Graphics pane (Fig. 17.37). Green bars in the ORFs display area represent individual ORFs. Click on a green bar to highlight the ORF in both the Graphics and Sequence Panes (Fig. 17.38).

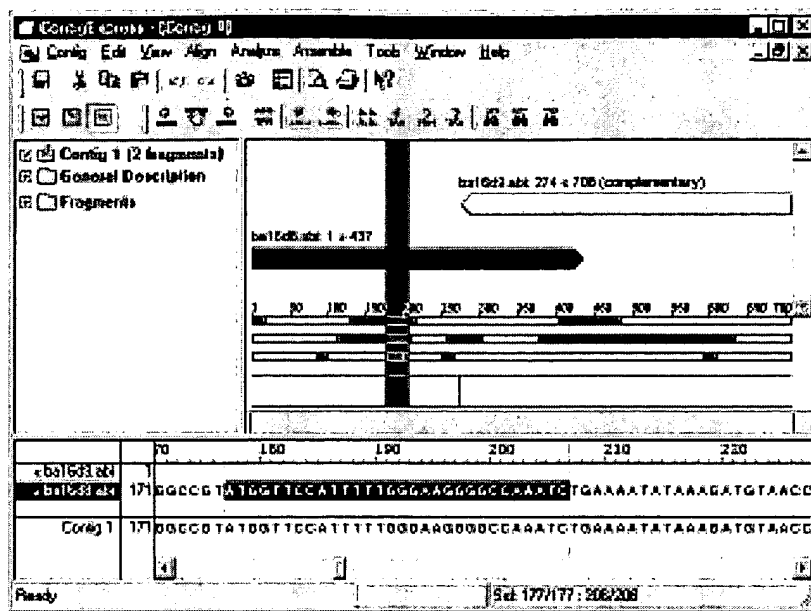


Fig. 17. 38 Clicking on a green bar highlight the ORF in both the Graphics and Sequence Panes

Parameters for the ORFs search can be configured in the Contig View Options dialog box. Choose **View > View Options** from the menu in the Contig Window to launch the dialog box. Select the **ORF** tab on the Contig View Options dialog box (Fig. 17.39).

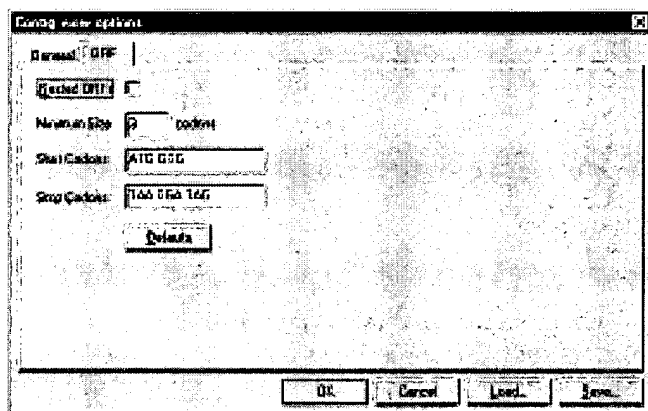



Fig. 17. 39 Contig View Options dialog box

Acceptable start and stop codons can be designated here. Minimum ORF size for the search can also be set, as well as the option to show nested ORFs.

2. Review the Contig in Contig Alignment Pane

Click the **Alignment** button () in the Window Toolbar to activate the Contig Alignment Pane (Fig. 17.40). The Alignment Pane displays the nucleotide sequences of fragments forming the contig and the contig itself with respect to the positions of the fragments in the contig.

Let's study the elements of the Alignment Pane:

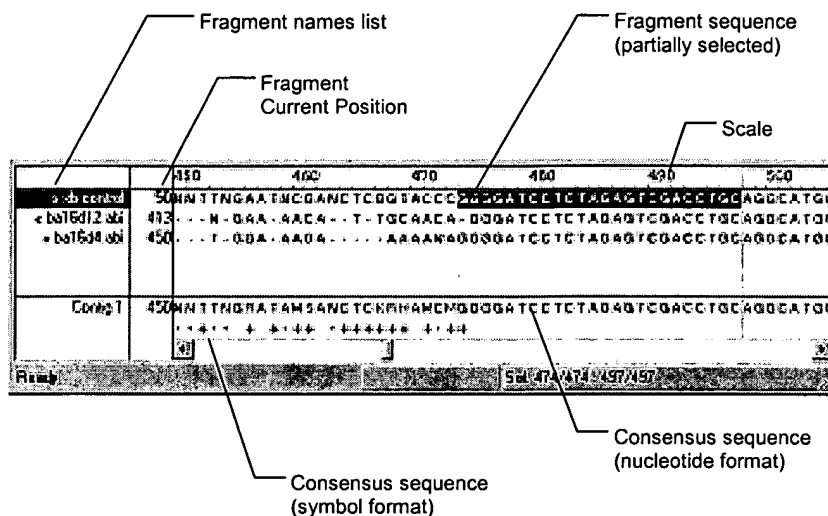



Fig. 17. 40 Contig Alignment Pane

- The **fragments names list** contains all fragments used in this contig
- The **scale** gives a reference for all fragments and the consensus shown in the alignment
- The **fragment current position** is the relative (counted from the fragment start) position of the currently shown left end of the respective fragment.
- The **consensus sequence (nucleotide format)** shows the nucleotide consensus for the assembly.
- The **consensus sequence (symbol format)** shows the symbol consensus for the assembly. In the symbol consensus, blank spaces indicate identical residues, dots (•) indicate positions containing Ns and plus symbols (+) indicate ambiguity and/or gaps.

A specific sequence fragment can be found in the Alignment Pane using the Find feature. Click on the **ba16d9.abi** fragment name in the Alignment pane list to select it. Launch the Find sequence dialog box either by choosing **Edit > Find** from the menu or by pressing the

Find button () on the toolbar. When Find dialog box appears, type in the sequence CAGTATATCCG (Fig. 17.41).

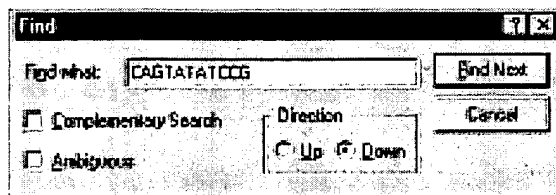


Fig. 17.41 Find dialog box

Press the **Find Next** button. The Alignment Pane displays the region from 419 to 429 bp where the sequence has been found; the found sequence is highlighted (Fig. 17.42).

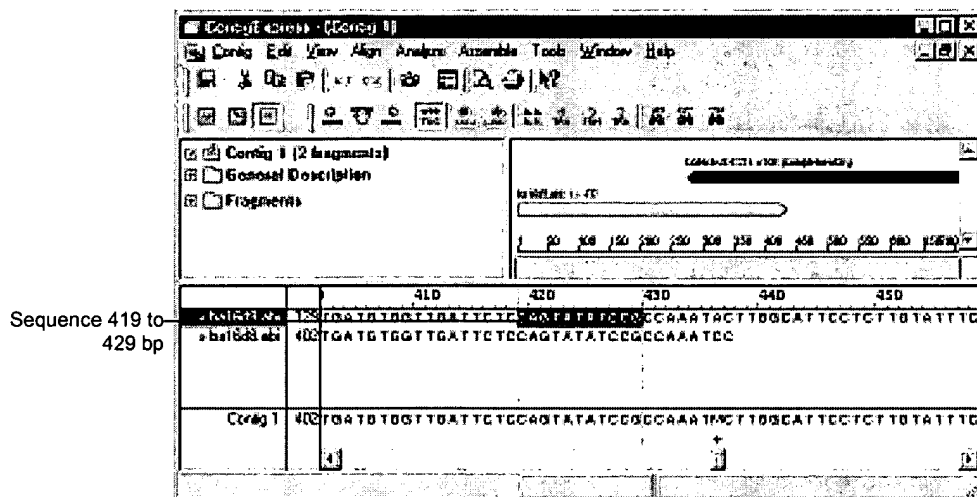


Fig. 17.42 The Alignment Pane displays the region from 419 to 429 bp

Click on the **ba16d8.abi** fragment name in the Alignment Pane. Right-click anywhere in the Alignment Pane, opening the shortcut menu. Select **Show Chromatogram for ba16d8.abi**. Select nucleotides 419 through 429. (Notice that selecting an area of sequence in the Alignment Pane also selects that area in the Graphics Pane.)

The chromatogram for the selected fragment is shown below its sequence, the same as it was in the Chromatogram Pane of the Fragment window (Fig. 17.43):

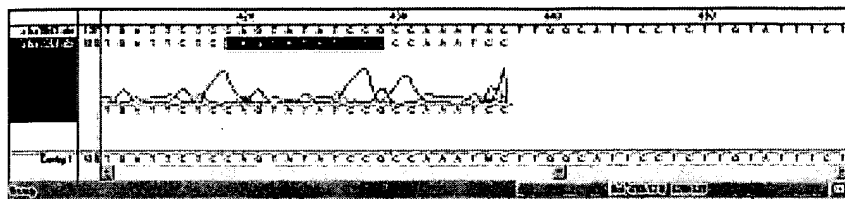


Fig. 17. 43 The chromatogram for the selected fragment is shown below its sequence

Right-click anywhere in the Alignment Pane. Select **Show All Chromatograms** from the shortcut menu. Now the chromatograms for both molecules are displayed (Fig. 17.44):

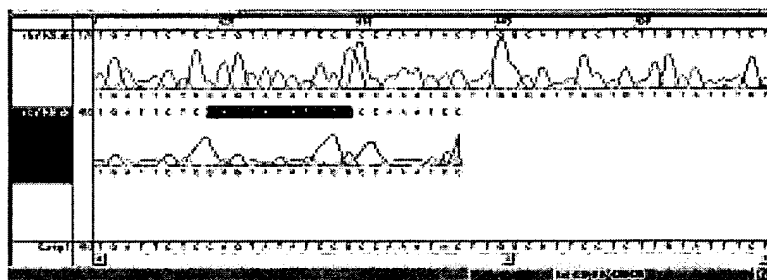


Fig. 17. 44 Using the Show All Chromatograms feature

Right-click anywhere in the Alignment Pane and choose **Hide All Chromatograms** from the shortcut menu. Chromatograms in the Alignment Pane are no longer displayed.

The consensus sequence can be translated in the Alignment pane in any one or all of the three direct frames. Translate the consensus sequence in the first frame by activating the Alignment Pane and choosing **View > Consensus Translation > 1st frame** from the menu or by pressing the **Consensus Translation 1st Frame** toggle button on the toolbar. Translate the consensus sequence in the second and third frames by choosing their respective menu commands or toolbar buttons (Fig. 17.45).

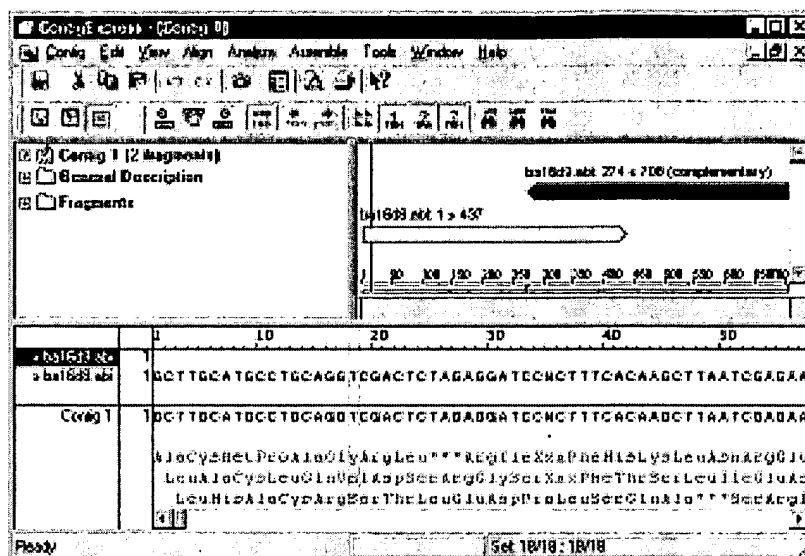


Fig. 17.45 Translating the consensus sequence

Translations can be removed by pressing the **Consensus Translation** buttons again or by deselecting the translation options from the **View > Consensus Translation** menu.

3. Edit a Fragment in the Alignment Pane

Fragments can be edited in the Alignment pane of the Contig Window. Make a selection from 419 to 429 bp and select **Edit > Delete Sequence**. Because this fragment takes part in some other assemblies besides the current one, a warning appears (Fig. 17.46):

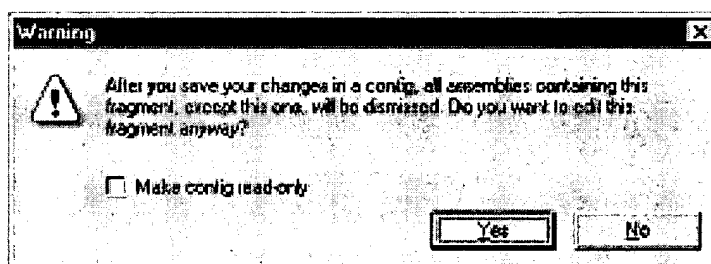


Fig. 17.46 Warning regarding deleting a sequence

Click Yes to accept the changes. *If you check the **Make contig read-only** box, the Yes option is disabled.* When you proceed with the deletion, the bases are deleted from the chromatogram and the gap is closed. A vertical bar appears in the sequence marking the position where the deletion was made (Fig. 17.47). The consensus is changed in that bases to the right of the deleted region now move to the left, filling the gap.

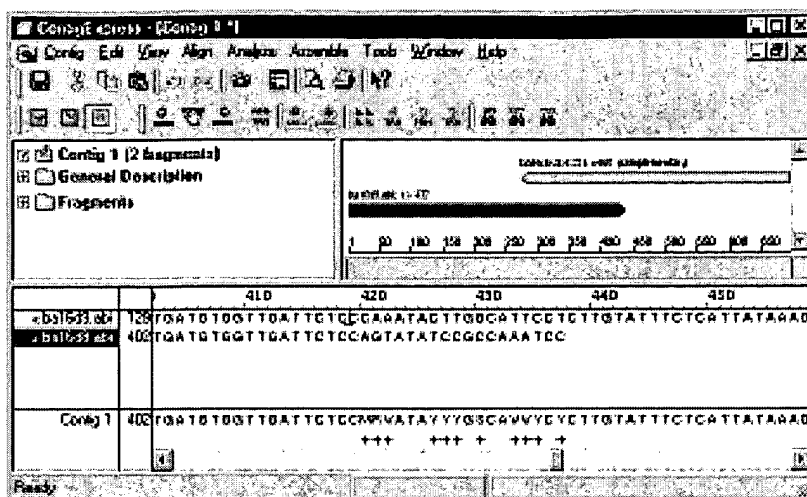


Fig. 17. 47 A vertical bar appears in the sequence marking the position where the deletion was made

If you save the edited contig and open the edited fragment in the Fragment Window, the editing changes are reflected in the fragment sequence.

Undo/Redo functions can be used to revert or repeat the changes you make. Click the **Undo** button until it becomes disabled and the fragment and the consensus return to their original state.

Select bp 35 "N" and type T. The "N" is replaced in both the fragment sequence and the consensus sequence (Fig. 17.48).

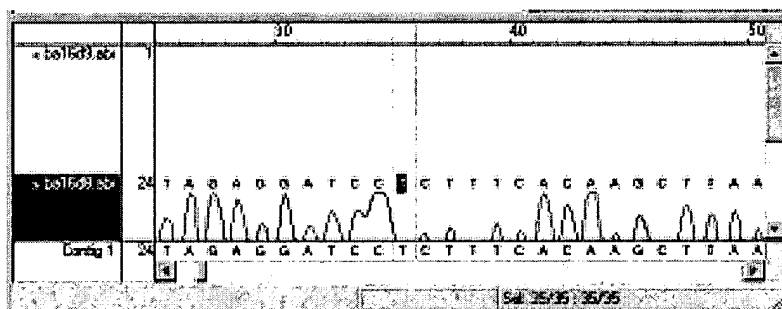


Fig. 17. 48 Replacing the fragment sequence and the consensus sequence

Once a fragment has been edited in the Contig Window, fragments can be realigned as needed by either saving the contig and reassembling it or by using the **Move Fragment** buttons on the toolbar to manually slide the fragment into place.

The only limitation of editing in the Contig window is that it invalidates other assemblies containing the newly edited fragment and causes those assemblies to be dismissed.

Fragment in-place editing and update of the consensus on the fly makes the Contig Alignment pane a convenient tool for working with contigs after their initial assembly.

Note: Once editing has been performed in a Fragment/Contig Window, the edits must be saved in both the Fragment/Contig Window AND the CE Project Window to ensure that the changes are retained.

4. Exit ContigExpress

Now you have finished the ContigExpress tutorial.

Close ContigExpress as you would close any other Windows application.

Chapter 18 Database Explorer

Introduction

The Vector NTI database is a collection of molecules and laboratory data, organized for easy retrieval and management in Vector NTI's Database Explorer. Seven different types of objects are stored and organized in databases and subbases in Database Explorer:

- DNA/RNA molecules are annotated with Feature maps. Upon import from other sources, molecule data is parsed and stored in an internal format. Users can add molecules to the database through import or creating basic or constructed molecules.
- Protein molecules are also annotated with Feature maps. Like DNA molecules, upon import from other sources, protein molecule data is parsed and stored in an internal format. Users can add molecules to the database through import or creating basic molecules.
- Restriction enzymes are imported from the REBASE database; data for the 229 enzymes in the Vector NTI database is parsed and stored in an internal format. Users can add other restriction enzymes from the REBASE file included in the Vector NTI software.
- Oligonucleotides in the database are user-defined except for several included in the Vector NTI software for demonstration purposes.
- Gel markers commonly used in molecular biology laboratories are included initially in the Vector NTI software, but new gel markers can be created by the user.
- Citations for physiochemical analysis of molecules in BioPlot and other Vector NTI analyses are stored in Database Explorer.
- BLAST results can be stored independent of molecules in a BLAST Results database.

Very similar in functionality to the Windows 95/98/Windows NT interface, Database Explorer supports intuitive browsing of local and shared Vector NTI databases, clipboard data exchange, drag and drop operations and other functions typical of window-based database management.

From the Exploring Database window, you can perform the following:

- Create new molecules
- Sort, Edit and delete molecules
- Search the database for text sequence, motifs, feature types, keywords etc.
- Organize our data into convenient groups (subbases)
- Import and export data (this is inactive in the demo version)
- Open the other applications of Vector NTI Suite—AlignX, BioPlot and ContigExpress.

Parent-Descendant relationships (to keep track of your constructs), user fields, comments, keywords etc., are kept for all molecules in the database

All database molecules and other objects can be placed into “archives”—data files of special format—that can be transferred to another computer (Mac or PC) and read by Vector NTI there. Through archives, you can share molecules, constructs, or other objects with your colleagues, or use them simultaneously on several computers (for instance, at work and at home).


In the Vector NTI archives:

- All DNA/RNA molecule information is written to and read from an archive file. This includes molecule component fragments (if the molecule is constructed from other molecules) and parent-descendant connections between molecules.
- Vector NTI automatically checks the consistency of molecule archive information adding necessary parents (including DNA parents of translated protein molecules) or disconnecting them if you have neglected to transfer them to the archives.
- When the archive is loaded into a new database, Vector NTI checks the information consistency on any of database molecules and recalculates them if necessary.

Important: The Vector NTI Database is completely independent of the Xpression NTI Database, and each database has its own unique viewer. It is not possible to exchange objects between the Xpression NTI and Vector NTI applications.

Opening Vector NTI Database Explorer

The Vector NTI Database Explorer can be opened from several sources:

- Database Explorer opens automatically in the Vector NTI workspace every time you initiate a Vector NTI session.
- Database Explorer can be opened and used independently by selecting the Database Explorer in the Vector NTI Suite options under **Start > Programs** on your computer.
- From a Molecule Display window, press the **Local Database** button () to open Database Explorer.

In this User's Manual, the term “Explorer” refers to Vector NTI Explorer, not to Windows Explorer.

Elements of Vector NTI Database Explorer

The Database Explorer window consists of a menu bar, a toolbar, Table drop-down menu in the upper left corner, a Subbase Pane on the left and a Database <Object> Pane on the right of the window (Fig. 18.1).

right pane (activating the Object Pane), the menu bar displays object-related menu options and toolbar buttons.

- To alternate between panes, click on a pane to activate it or use the F6 keyboard shortcut. To open context-sensitive shortcut menus, click on subbases or database objects with a right click. The shortcut menu displays commands appropriate for the object under the cursor.

Database Objects

Each database object is described by a set of fields, describing various object data. Each object type defines its own set of fields: System fields are common for all database objects; other fields are specific for a particular object type. A user may also define additional user fields.

Database Object System Fields

Most of the object system fields, assigned names by Vector NTI, are accessible from various parts of the program's user interface. Some of them, however, are only visible in specialized object type-specific dialog boxes or display windows.

Here is the full list of named system fields displayed by Vector NTI:

<i>Field name</i>	<i>Description</i>	<i>Used in: N(DNA/RNAs) P(proteins) E(enzymes) O(oligos) G(gel markers) C(contacts) U(user field defs)</i>
Name	Name of an object	NPEOGCU
Local/Foreign	Object's 'ownership' status in the local database	NPEOG (local DB only)
Original Author	Original author (creator) of an object	NPEOG
Created	Date of an object's creation (See Date and Time section following)	NPEOG
Author	Author of the last modification to an object (see following section)	NPEOG
Modified	Date of the last modification to an object	NPEOG
Submitted by	The name of a Vector NTI user who submitted the object	NPEOG (shared DB only)
Description	Description string	NPEOGU
Form	Circular or Linear	N

Field name	Description	Used in: N(DNA/RNAs) P(proteins) E(enzymes) O(oligos) G(gel markers) C(contacts) U(user field defs)
Storage Type	Basic, Designed or Constructed	NP
Fully Processed?	Molecule's construction status	N
Length	Length of a molecule/oligo/enzyme's recognition string in bp	NPOE
DNA/RNA	Nucleic acid type	NO
Degenerate?	Is an oligo degenerate?	O
Oligo Sequence	Oligo's nucleotide sequence	O
Recognition String	Enzyme's recognition string	E
Terminus Type	Enzyme's terminus type	E
Palindromic?	Is an enzyme palindromic?	E
Ambiguous?	Is an enzyme ambiguous?	E
Number of Fragments	Number of fragments in a gel marker	G
Longest Fragment	Length of the longest fragment in a gel marker	G
Shortest Fragment	Length of the shortest fragment in a gel marker	G
Telephone	Phone part of the Contact info	C
E-mail	E-mail part of the Contact info	C
Field Type	User field type	U
Indexed?	Is user field cached/indexed?	U
Default	Default value of a user field	U
List of Predefined Values	List of predefined values of a user field	U
Record #	Database record ID number	NPEOG

Table 18. 1 Named system fields displayed by Vector NTI

In addition to the system fields named above, every database object has comments and keywords associated with it. These fields are accessible from within specialized object editors and Display windows.

Note: All automatically updated fields, user-defined fields and comments are searchable. Using the database search interface described later in this chapter, you can perform string context searches through any of the fields above for any database objects.

Of the general system fields data itemized in the above table, only Author and Date and Time display features are independent of object types. Their sources and modification options are summarized below.

Author Information

Information regarding authors and times of creation and modification for all database objects is stored in the following named System Fields:

- **Original Author:** name of Vector NTI user who created the object,
- **Author:** name of Vector NTI user who last modified the object,
- **Creation Date:** date and time when the object was created,
- **Modification Date:** date and time when the object was last modified in the local Vector NTI database.

When you start Vector NTI for the first time, in the Author Information dialog box you can identify yourself as Vector NTI user and author of all modifications and new objects you create in the local database. By default, it presents the name given to the Vector NTI Installation program. This information is automatically attached to all database objects you create and/or modify when you export them, copy to shared databases etc.

Note: Changes you might make later to Author Information do not affect information stored in database objects you submitted to shared databases earlier.

- An object you create in your database is marked as a “Local Object” naming you as both the author and the original author. While author information is not displayed on local objects while you view or edit them, the data does follow the object and is displayed when exported or viewed by another VNTI user.
- When you import an object or transfer it from a VNTI Shared Database into your local database, if you are the author the object is still marked as Local. If the author is someone else, then the object is marked as “Foreign” and the author information is always displayed when you are viewing or editing the object.
- The original author (creator) of an object, either “Local” or “Foreign”, never changes. If you modify a “Foreign” object, however, it becomes “Local” and you become its new “immediate” author.

- If you are importing an object in which author or original author information is not stored in your contact database, VNTI lets you update the contact and author data in your database.

To review or edit author data, select **View > Author Infor...** opening the Author Information dialog box where you can do that.

Date/Time Display

To change the date/time display format, select **Edit > Options** on the menu bar of the main Vector NTI's window. On the: General tab of the Options dialog box, select the standard US date format (for example, 03/20/96 04:20PM) or a regional date/time format, the default format recognized by your computer. Change this format using the Windows Control Panel/Regional (or International) dialog box.

Authors, original authors, and times of creation and modification for all database objects are updated automatically by Vector NTI and should not, in normal circumstances, be modified manually. If such a need occurs, use the <database object > Set System Attributes command for setting values of these fields in the Vector NTI Database Explorer.

User-defined Fields

For information management functions, user-defined fields are associated with any database object (molecules, oligos, etc.). A User Field containing laboratory-specific and user-specific descriptions can be imported, exported, displayed, printed and managed just as the regular database fields.

While previously defined User Fields are maintained and presented when you create new database objects, imported objects may contain fields not described on the local list. Vector NTI helps you to add their descriptions to the list whenever you try to edit the information contained in these fields. Vector NTI also allows you to import and export User Field Definitions across several VNTI databases.

The “User Fields Manager” section on page 251 of this chapter contains the detailed description of all User Fields-related operations. For a detailed example of how to use the User Fields Manager, walk through the Database Tutorial in Chapter 8.

Comments

Each database object can contain a text comment of unlimited length. Vector NTI automatically converts stored comments when importing or exporting molecules from/to GenBank/GenPept and EMBL/SWISS-PROT files.

Comments can be edited in the Comments tabs of the objects' editors or immediately in the Molecule Display window (for molecules only). In a Molecule Display window, double click on the Comment line or click on the Comment icon to open the Comment Editor containing the molecule comment.


Keywords

Each database: object can contain a list of associated keywords that are very useful for database searches. Keywords can be edited in the Keywords tabs of the object's editors.

To enter keywords, in the keywords text box, type a new word or select an item from the list of existing keywords. Press the **Add** button to move the keyword into the keyword list. To remove an item from the list of keywords, select the item(s) you want to remove and press the **Remove** button.

Database Search

You can perform a database search to locate any type of object stored in the database. In general, search procedures launched from Database Explorer follow these basic steps:

1. Select the appropriate Database Table type and select **Database > Search** on Explorer's menu bar or click the **Search** button () on the toolbar.
2. In the <Object > Database Search dialog box, verify the Database Table type.
3. In the Search dialog box, (Fig. 18.2) check the boxes for the filters to be included in the search. Press the enabled filter buttons to open dialog boxes.
4. In the Conditions Filter dialog boxes that open, define additional search parameters. (See detailed descriptions of filters below.) *Only those molecules that satisfy all the conditions you define are found in the search.* Click **OK** in each to return to the Search dialog box.
5. When you are satisfied with the search setup, press the **Start Search** button.
6. If Vector NTI finds any objects by a database search, the appropriate Subbase Name dialog box appears. Enter a name of new subbase or choose a subbase name from those listed. All objects found will be placed in the specified subbase overwriting the existing subbase content (if any).

Note: If the specified subbase already exists, Vector NTI empties it before adding found objects. You can refine your search by using the resulting subbase to limit the search space of the next search.

Database search filter parameters specific to particular database objects are outlined below. Specific settings are not discussed unless they need clarification.

DNA/RNA and Protein Molecule Database Searches

The Molecule Database Search dialog box (Fig. 18.2) for DNA/RNA and protein molecules are identical except for the database shown in the Look In drop-down menu. Differences in the specific filter setup dialog boxes are specified in the discussion for each.

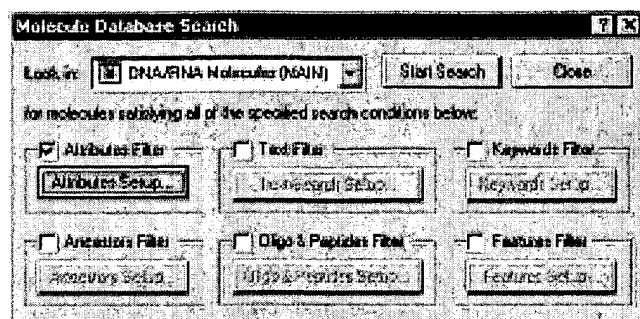


Fig. 18. 2 Molecule Database Search dialog box

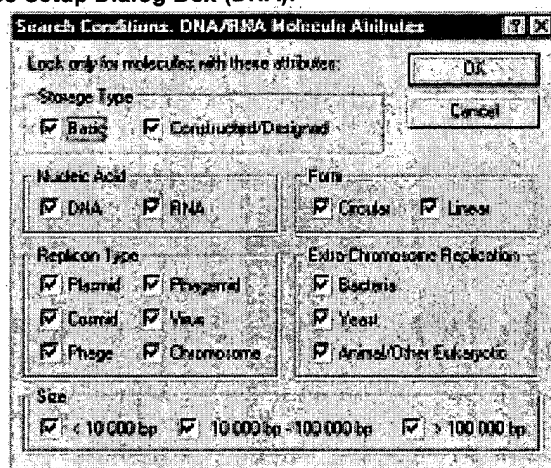
Attributes Setup Dialog Box (DNA):

Fig. 18. 3 Attributes Setup dialog box (DNA)

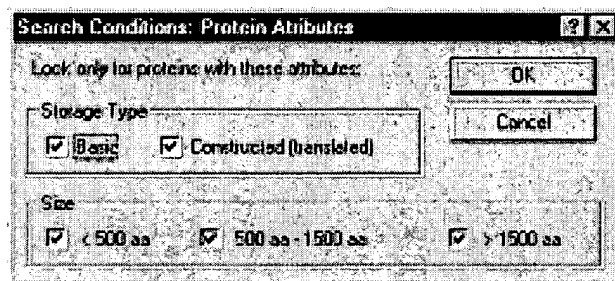
Attributes Setup Dialog Box (Protein):

Fig. 18. 4 Attributes Setup dialog box (Protein)

If all conditions in a group are checked, or if all conditions in a group are blank, that group has no effect on the search.

Text Setup Dialog Box (Identical for DNA and Protein):

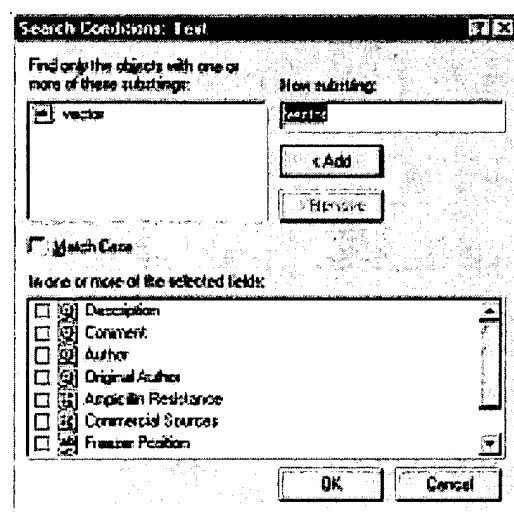


Fig. 18. 5 Text Setup dialog box

To add a new substring, enter the text and click the **Add** button (Fig. 18.5). To remove a substring, highlight it and click the **Remove** button. Check the fields to be searched for the text substrings. Check **Match Case** for case-sensitive text substrings.

Keywords Setup Dialog Box (Identical for DNA and Protein):

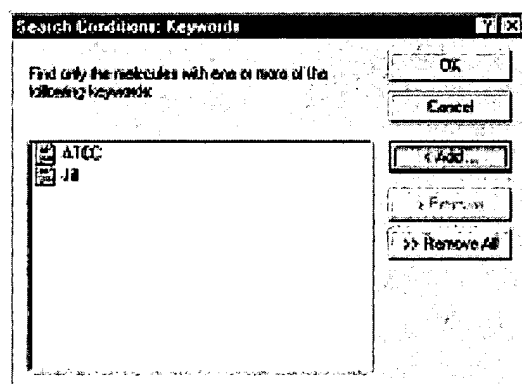


Fig. 18. 6 Keywords Setup dialog box

Press the **Add** button to select from the list of existing keywords (Fig. 18.6). To remove a keyword from the box, select it and press the **Remove** button. To clear the Keywords box, press the **Remove All** button. **Note:** You cannot add keywords in this dialog box.

Ancestors Setup Dialog Box (Identical for DNA and Protein):

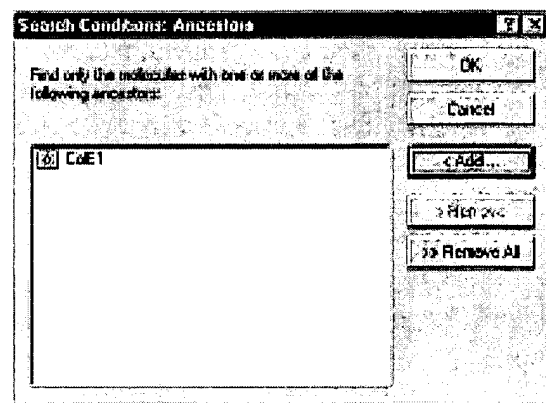


Fig. 18. 7 Ancestors Setup dialog box

Molecules that have as a direct ancestor any one of the molecules shown in this dialog box will be found by the search. *Ancestors for proteins in this context are DNA molecules.*

To add a molecule to this box, press **Add**. Select from the database molecules list and click **OK**. To remove a molecule from the ancestors list, select the molecule in the dialog box and press **Remove**, or to clear the list, press **Remove All**.

Oligo & Peptides Setup Dialog Box (DNA and Protein):

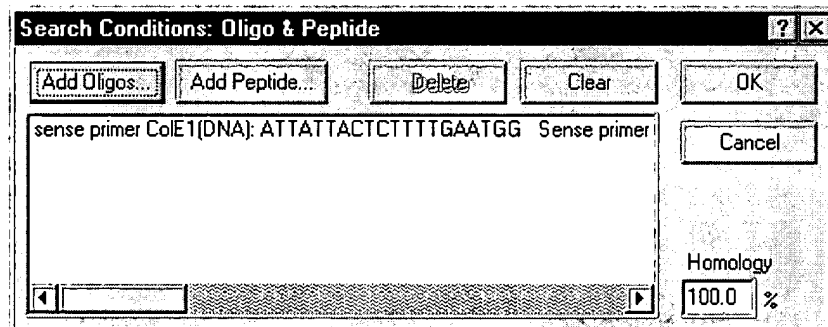


Fig. 18. 8 Oligo & Peptides Setup dialog box

To search in the DNA/RNA database, to add an oligonucleotide for similarity search, press the **Add Oligos** button. To add a peptide for similarity search, press the **Add Peptide** button. In the dialog box that opens, enter the name and appropriate query sequence, as

prompted, for the database search. (For a protein database search, only the **Add Protein** button is enabled.)

To remove a search condition from the list box, select the condition and press **Delete**. To clear the Oligonucleotides/Peptides box, press **Clear**.

Feature Setup Dialog Box (Identical for DNA and Protein):

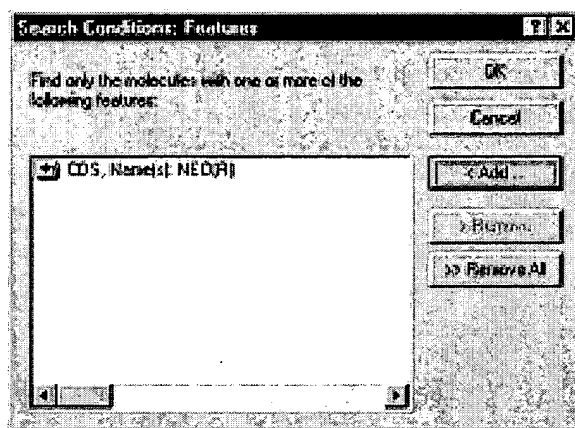


Fig. 18. 9 Feature Setup dialog box

To add a feature to the search conditions, press the **Add** button (Fig. 18.9). In the dialog box that opens (Fig. 18.10), define restrictions for the molecule search. *Feature types differ for DNA/RNA searches and protein searches.*

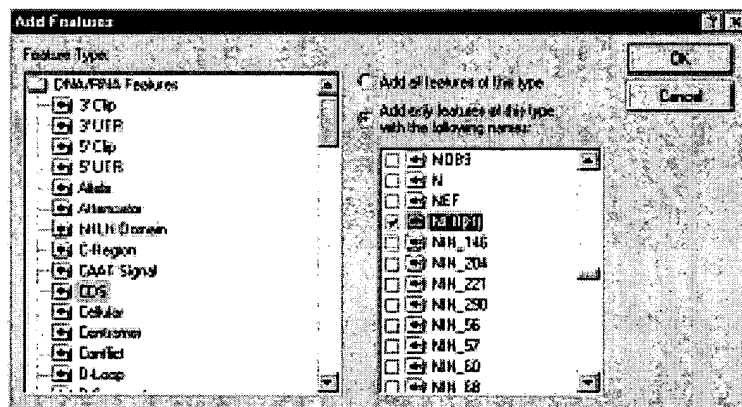


Fig. 18. 10 Add Features dialog box

Select a feature type in the left pane and optionally, using the radio button, one or more feature names. If you define a type of feature but no specific feature names, the search condition is defined as any feature of that type.

To remove a search condition from the Features list, select the condition and press **Remove**. To clear the Features list, press **Remove All**.

Enzyme Database Search

When you launch an enzyme search, the following Search dialog box opens (Fig. 18.11):

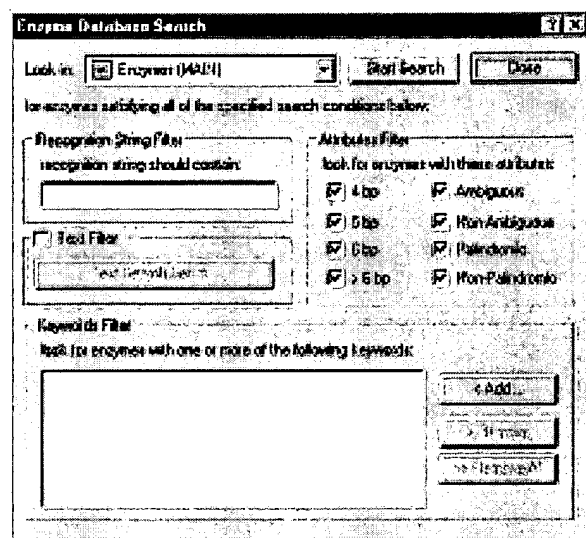


Fig. 18.11 Enzyme Database Search dialog box

- **Recognition String Filter:** Enter a nucleotide string that must be found in the recognition site of the desired enzyme.
- **Attributes Filter:** Specify enzyme attributes necessary for the search. “Ambiguous” recognition site means the site containing any nucleotide besides A, T, G, or C.
- **Text Filter:** Check the **Text Filter** button to filter search results based on text inside various object fields. Refer to page 224 for a description of the Text Filter Condition.
- **Keywords Filter:** Refer to page 225 for a description of the Keywords filter. **Note:** You cannot add keywords in this dialog box.

Oligo Database Search

When you launch an Oligo database search, the following dialog box opens (Fig. 18.12):

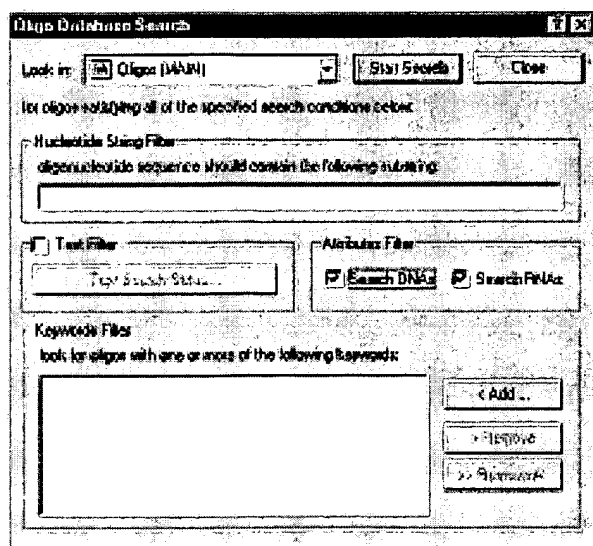


Fig. 18. 12 Oligo Database Search dialog box

- **Nucleotide String Filter:** Enter a Nucleotide String filter that must be found in the sequence of the desired enzyme.
- **Attributes Filter:** Check to limit the search space to DNAs only or RNAs only. If both are checked or unchecked, the filter has no effect.
- **Text Filter:** Check the **Text Filter** button to filter search results based on text inside various object fields. Refer to page 224 for a description of the Text Filter Conditions.
- **Keywords Filter:** Refer to page 225 for a description of the Keywords filter. **Note:** You cannot add keywords in this dialog box.

Gel Marker Database Search

When you launch a search in the Gel Marker Database, the following dialog box opens (Fig. 18.13):

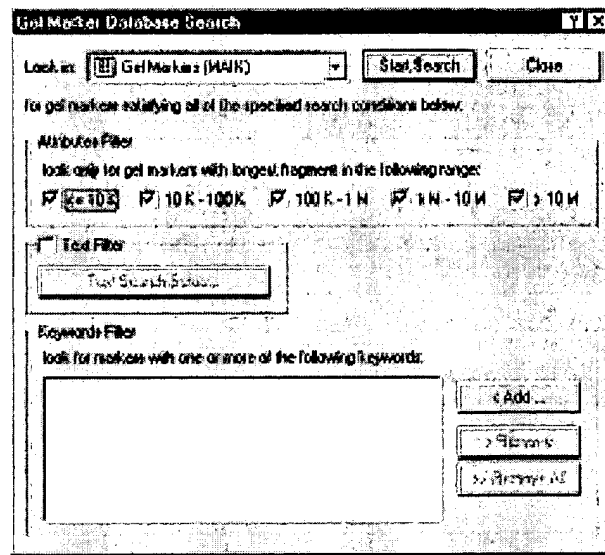


Fig. 18. 13 Gel Marker Database Search dialog box

- **Attributes Filter:** Check to limit the search space to gel markers with specified length of the longest fragment. If all are checked or unchecked, the filter has no effect.
- **Text Filter:** Check the **Text Filter** button to filter search results based on text inside various object fields. Refer to page 224 for a description of the Text Filter Condition.
- **Keywords Filter:** Refer to page 225 for a description of the Keywords filter. **Note:** You cannot add keywords in this dialog box.

Citation Table

The Citation Table in Database Explorer is a convenient vehicle in the database for storing citations retrieved through the Vector NTI PubMed Search engine, from the NCBI website or from other searches. In addition, the Citation Table section of Database Explorer provides complete bibliographic conversion features described below.

One of the most convenient features of the Citation Table is its ability to generate citations and bibliographies for thesis, dissertations and other publications. Bibliographies are generated according to the rule of scientific journals.

Formatting Bibliographic References

To format each bibliography record, you must insert a tag in the document where the reference is to be cited. In Database Explorer Citation Table, select the desired reference. From its associated shortcut menu, select the **Copy Tag** command. Then go to your word processor document and paste it. For example, the tag at this point might read [Smith, J. 1994 #213].

After finishing the writing, save your document as Rich Text Format (*.rtf) file.

When you are ready to prepare your manuscript, select the Citations Table in Database Explorer. Open a shortcut menu in the subbases pane and select **Format Manuscript**. In the dialog box that opens (Fig. 18.14), browse for the RTF file you want to format.

In the Format Reference For drop-down menu, select a journal name. Click the **Start** button. The tagged document will be formatted into the document with properly formatted citations and bibliographies.

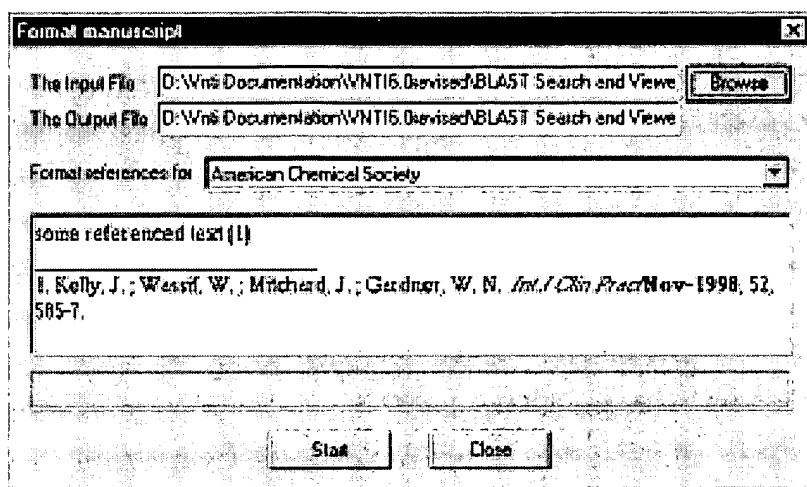


Fig. 18. 14 Format Manuscript dialog box

To place selected citations onto the Clipboard formatted according to the rules of specific journal, select the citation(s) and open the shortcut menu in the Database Objects pane. Select **Copy as Bibliography**. In the dialog box that opens (Fig. 18.15), select the journal whose formatting the bibliography should follow. Click **OK**.

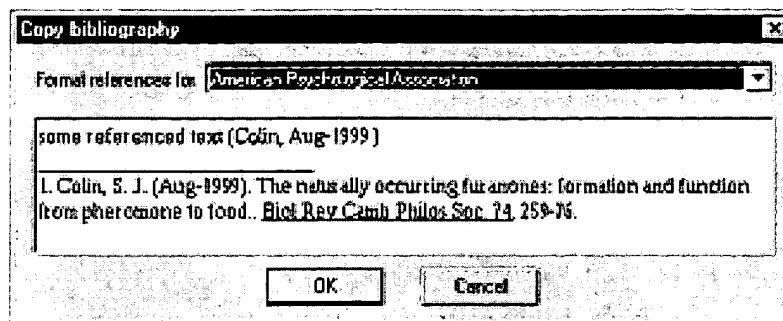


Fig. 18. 15 Copy Bibliography dialog box

Then proceed to your word processing document and paste the citations. They will be in the format for the journal you selected.

Note: In the Database Explorer, one cannot edit citations downloaded from NCBI.

BLAST Results Table

The BLAST Results table is a repository for BLAST results you wish to store. The subbases and objects are managed and manipulated exactly as with other database subbases and object described elsewhere in this chapter.

To open BLAST results from the Database Explorer, double-click on the selected results in the Database Objects Pane.

Database Management Operations

Backup, Restore and Cleanup

Three general functions (all initiated by choosing options under the Database menu of Database Explorer) relate to the entire database:



- **Database Backup** makes a copy of your database in a specified directory or folder. For instance, you may back up the data to removable storage to make a permanent, safe record of your database. Making such a backup is the *only* way to be sure your data is safe in the case of hard drive failure.
- **Database Restore** restores all database files from the last backup. Your database is restored from a specified directory or folder, overwriting all current database files.
- **Database Cleanup** removes from the data files and database tables all the “garbage” data that may accumulate when you extensively create and delete database objects. This also serves as a recover function when files or tables are lost or damaged. Although these data do not affect Vector NTI functionality, it is wise to perform Database Cleanup once a week.

Operations on Subbases

To initiate any of the following operations on a subbase, select the subbase name in the left pane of Database Explorer. Press the toolbar buttons as described or choose Table or Edit or corresponding shortcut menu commands. Descriptions of each operation are included below.

Creating a New Subbase

To create a new subbase and/or add objects to it, follow one of the following options:

- Select **Table > New > Subbase** or click the **New Subbase** button () to create a new (empty) subbase with a default name, Group 1. You can edit this name in the enabled text box; press enter to confirm.
- Select **Table > New > Subbase (Using Subbase Editor)** to create a new subbase and select its contents using the Edit Subbase dialog box described below under Editing a Subbase.
- A simple way to add new molecule or other object to a subbase is to open the source subbase in the Subbase Pane. Select and drag selected objects from the list to the new subbase. The objects are now listed in the original subbase and the new subbase.
- To create a new subbase and place objects in it at the same time, select objects for the new subbase in the Database Objects Pane and click the **New Subbase** button ()

Editing a Subbase

In the Subbase Pane, select a subbase to be edited and select **Table > Edit Subbase**. *This option is not available for subbases where the content is maintained automatically.* This opens the Edit Subbase dialog box (Fig. 18.16):

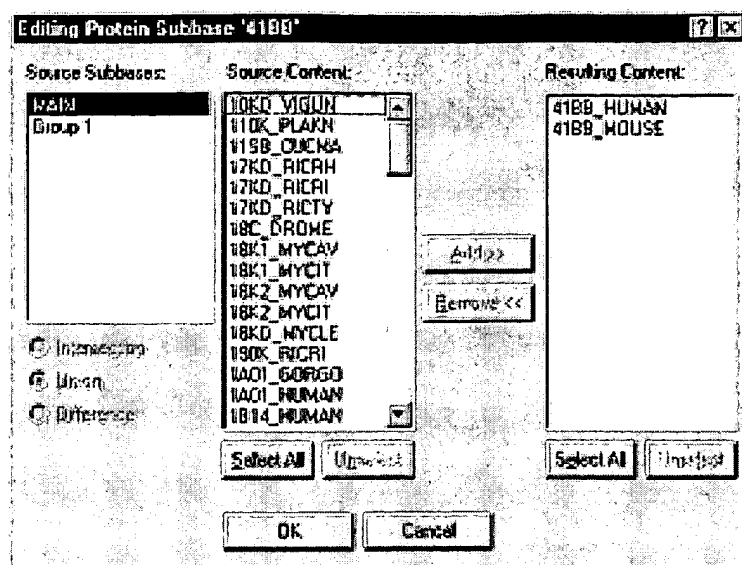


Fig. 18. 16 Edit Subbase dialog box

- The Source Subbases column shows all of the subbases in your Vector NTI database table **except** the one you are editing. The buttons below this column control how subbase objects in subbases selected in the Source Subbases column are displayed in the Source Content column:
 - **Intersection:** Each molecule that is present in all highlighted subbases is displayed in the Source Content column. Molecules not included in all selected subbases are thus excluded.
 - **Difference:** Each unique molecule from selected subbases, in other words, a molecule present in only one of the highlighted subbases is displayed. Molecules that occur in more than one subbase are thus excluded.
 - **Union:** All molecules that is present in any of the selected subbases is displayed.

If only one subbase is highlighted, the **Intersection**, **Difference**, and **Union** buttons are disabled, and all the objects in that subbase are shown in the Source Content column.

- The Source Content column shows combinations of objects present in the subbases highlighted in the Source Subbases column. *See descriptions under the previous bullet.*
- The Resulting Content column shows the contents of the subbase you are editing.

To add objects to a subbase, highlight one or more subbases in the Source Subbases Column. In the Source Content column, highlight the object names or use the **Select All** and **Unselect All** buttons in that column to highlight or deselect all objects at once. When the


objects you want are highlighted, press the **Add** button to add them to the Resulting Content column. Click **OK** to exit the dialog box. The objects are now listed in the original subbase and the new subbase.

Removing Molecules from a Subbase


In the Subbase Pane, select the subbase to be edited. Select **Table > Edit Subbase** or **Edit** from the shortcut menu. The Resulting Content column in the Edit Subbase dialog box (Fig. 18.16) shows the current contents of the subbase you are editing. To remove objects from the subbase, highlight their names in the Resulting Content column and press the **Remove** button. **Select All** and **Unselect All** buttons below the Resulting Content column allow you to highlight or deselect all objects in the column. Click **OK** to exit the dialog box. Database objects that are removed continue to exist in the database and other subbases where they were included before this operation.

For alternative ways of removing objects from a subbase, see Operations on Database Objects on page 238.

Rename Subbase

Select **Table > Rename Subbase** or press the **Rename** button (). In the enabled text box, edit the name. This operation does not affect the subbase contents or database objects. *This option is not available for subbases where the content is maintained automatically.*

Dismiss Subbase

Select **Table > Dismiss Subbase** or press the **Dismiss Subbase** button (). This operation removes a selected subbase, but does not affect the database objects. They are still stored in the database and exist in other subbases where they were included before this operation. *To empty the subbase first, use the Clear Subbase operation.*

Clear Subbase

Select **Table > Clear Subbase** to exclude all objects from the selected subbase. The subbase itself is emptied but not removed. This operation does not affect database objects; they are still stored in the database and included in all other subbases where they were included before this operation. *This option is not available for subbases where the content is maintained automatically.*


Delete Contents

Select **Table > Delete Contents from Database** to physically delete from the database all objects of the specified subbase. If you choose **OK** in the confirmation dialog box, the objects in the subbase are permanently deleted from the Vector NTI database, and the subbase itself is removed.

Subbase Summary

Select **Table > Subbase Summary** to create a text file containing a summary of the specified subbase. In the Write Summary To dialog box that opens, indicate the directory or folder and file name where the summary will be stored. Press the **OK** button and Vector NTI creates the summary.

Subbase Properties

Select **Table > Properties** or the **Properties** button () to display properties for the selected subbase.

Import Molecules into a Subbase

To import molecules into a subbase using *drag and drop*, use one of the following techniques:

- **Import Molecule(s) or Sequence(s) From Text File(s):** Highlight the molecule file name(s) in Windows Explorer (**Note:** molecule files must be in accepted formats, see below). Drag and drop the highlighted name(s) onto the desired subbase in the VNTI Database Exploring window.
- **Import Directory of Files:** For a folder containing multiple molecule files (in accepted formats, see below), drag and drop the folder onto the desired subbase in the Exploring Local VNTI Database window. All the molecules in the folder are imported into the subbase.

To import molecules into a subbase using *menu options*, select **Table > Import**, then one of the following options:

- **Import Molecule From Text File** imports DNA/RNA or protein molecule data from a GenBank/GenPept, EMBL/SWISS-PROT, or FASTA file. Vector NTI imports all molecule data including feature table (if any).
In the Import Molecule dialog box, choose the appropriate import file format—GenBank, EMBL, or FASTA for DNA/RNA molecules and GenPept, SWISS-PROT or FASTA for proteins and press **OK**. In the Import dialog box, browse for the source and file to be imported and click **OK**. Vector NTI reads the file, checks all data, creates the new molecule, and displays its data in the Molecule Editor dialog box. After entering or editing the data, press **OK**. Vector NTI saves the new molecule in the database.
- **Import Sequence From Text File** imports a nucleotide or amino acid sequence text file, creating a new DNA/RNA or protein molecule with the sequence. *This must be in ASCII format.* In Import dialog box, browse for the source and file to be imported and click **OK**. Vector NTI reads the file, checks all data, creates the new molecule, and displays its data in the Molecule Editor dialog box. After reviewing or editing the data, press **OK**, saving the new molecule in the database.

For ASCII format details, see Appendix C.

- **Import From Vector NTI Archive** imports objects from a Vector NTI archive. In the Import dialog box, browse for the source and archive file to be imported and click **OK**.
- **Import Objects From Directory of Text Files**, (option for molecules and oligos only), imports molecules and oligonucleotides from text files that must be in the GenBank format (DNA/RNA molecules), GenPept format (protein molecules) or Vector NTI's oligo text format (oligonucleotides). In the Import dialog box, browse for the List File and open. *When Vector NTI exports the objects into a directory of text files (see below), it automatically creates the appropriate List File.*

Notes on Molecule Import:

- In Vector NTI versions prior to 6.0, import and export functions could be performed from the Molecule Display window. In version 7.0, they can be performed only through Database Explorer.
- GenBank, GenPept and EMBL feature tables usually do not contain names for features. When importing, Vector NTI assigns "artificial" names for the features so that they will be recognizable on a graphics map. You can change features' names after import using Vector NTI's Molecule Editor, or assign names to features before import, by adding "label=<name > " descriptors in the flatfile for any desired features. Here is a sample GenBank/EMBL feature description with a defined feature name:

```
CDS    86..1276
      /label=TC (R)
```

For more information about GenBank/GenPept /EMBL Feature Table descriptors, see GenBank/GenPept /EMBL documentation.

Import Enzymes into a Subbase

Restriction endonucleases can be imported into Vector NTI from the REBASE database, which is available through anonymous ftp site, <ftp.neb.com>. To obtain further information refer to the website: <http://rebase.neb.com/rebase/rebase.html>. *For a recent review of the REBASE database see Nucleic Acids Research 22: 3628-3639, 1994.*

Each REBASE release contains several data files in different formats. Vector NTI uses Bairoch format, so you can download the bairoch.### file where ### indicates the current version (for example, 506 indicates June 1995). Distributive version of Vector NTI contains the last release of the REBASE bairoch.### file in the Vector NTI working directory.

To import restriction endonucleases into the Enzymes Table, select **Table > Import**, then one of the following options:

- **Enzymes From Archive:** Browse for the Archive file and open.
- **From REBASE Database:** Browse for **Vector NTI Suite > bairoch.###file** (see above) and select it in the Read REBASE Enzymes dialog box (Fig. 18.17). Click

To filter the enzymes in the Source Enzymes / Selected columns, check on or off the appropriate boxes in the **Use REBASE Subbase** group in the lower right corner of the dialog box and press the **Hide Others** button. This is particularly convenient when wish to identify restriction enzymes based on certain criteria.

When all the desired enzymes are in the Selected list, press **Import Selected** button to import them into Vector NTI.

Export Subbase

Export Subbase Into Vector NTI Archive


Select **Table > Export > Subbase into Archive** to export all objects of the specified subbase to a VNTI archive. In the File Save dialog box, indicate the directory or folder, and file name for archive storage. Press the **OK** button.




Export All Subbase Objects Into Directory of Text Files (molecules and oligos only)

Select **Table > Export > Subbase to Directory of Text Files** to export all objects of the specified subbase into a directory of text files in GenBank (DNA/RNA molecules), GenPept (protein molecules) or Vector NTI's oligo text format (oligonucleotides). In the dialog box, browse for the location and enter the file name under which the subbase is to be stored.

Operations on Database Objects

To perform various operations on an individual database object or group of objects selected in the Object Pane of the Database Explorer. Select menu options from the leftmost menu that corresponds to the currently displayed table type. Most of the following options appear with all database objects. Most commands are also available from the objects' shortcut menu. When available from toolbar buttons, they are shown. Menu commands specific for certain objects are discussed in the next section.

- **Open:** opens display windows for selected objects; available only for DNA/RNA and protein molecules.
- **Edit:** includes editing options for an object selected in the Database Objects Pane, such as user fields, comments, keywords, etc. *This command is enabled only with a single selected object.* You can also use the **Edit** button (.
- **New:** includes options for creating a new subbase for selected object(s) or creating a new molecule or object. For specific details, refer to creating new <specific object> in the index.
- **Import....:** operations are described in the previous section
- **Export:** operations are described in the previous section
- **Exclude from Subbase** Tree Pane History view excludes selected objects from the current subbase. Objects are still stored in the database and are included in all other subbases where they were included before this operation.

- **Delete from Database:** Physically deletes a selected object from the database. (Confirmation is requested before deleting the object.) *If a DNA molecule is deleted, Vector NTI scans all its descendants and disconnects them from the deleted molecule.*
You can also use the **Delete** button ().
- **Rename:** renames the selected object. If a DNA molecule is renamed, Vector NTI scans all its descendants and also changes molecule's name in all component fragment descriptions of all the molecule's descendants. You can also use the **Rename** button ().
- **Duplicate:** makes copies of the selected database objects. The replicas of selected objects are created in the database and included in the current subbase. The replica of an object named 'x' will be called 'Copy of x'. **Note:** *Copies of objects are not related to original objects. Thus, "copies" do not track further changes you may make to the originals.*
- **Properties:** displays the properties of the selected object(s). If one object is selected, all the named object fields with their values are displayed. Some object data (like sequence and comments) are not stored in named fields and are not displayed in the properties dialog box. For molecules, the property dialog box has two extra tabs: Parents and Descendants, displaying Parent and Descendant trees for the selected molecule. You can also use the **Properties** button ().
- **Set System Attributes:** Allows manual modification of the automatically updated fields of the selected objects - Author, Original Author and Creation Date in the Set System Attributes dialog box. If checked, the drop-down menu (edit box in case of Creation Date) below the check box is activated for setting the value of the appropriate field.

The Author and the Original Author fields can be set only with the names of contacts already stored in the contact database. The Creation Date field must be set with a date and time in the USA format: "MM/DD/YY H:MIN AM(or PM)" where H is from 1 to 12. For more information refer to pages 220-7.

DNA/RNA Molecule Operations

These operations are applicable to DNA/RNA molecules only:

- **New Molecule Using Sequence Editor** creates a new DNA/RNA molecule, entering the nucleotide sequence manually using Sequence Editor. The Molecule Editor dialog box is described in Chapter 20.
- **Delete with Descendants from Database** physically deletes the selected DNA molecule together with its descendants (DNA/RNA and protein molecules) from the Database. Confirmation is requested before deletion.

Protein Molecule Operations

This operation is applicable to protein molecules only:

- **New Molecule Using Sequence Editor** lets you create a new protein molecule, entering the amino acid sequence manually using Sequence Editor. The Molecule Editor dialog box is described in Chapter 20.

Enzyme Operations

These operations are applicable to RENs (restriction endonucleases) only.

- **New Enzyme** opens the Enzyme Editor tabbed dialog box where you can create a new database enzyme in the Enzyme Editor (Fig. 18.19):

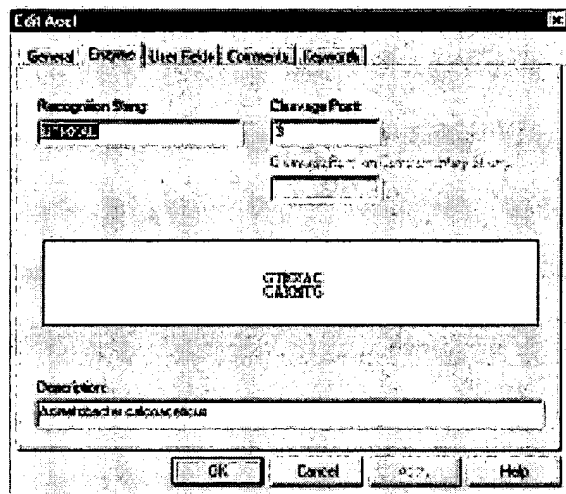
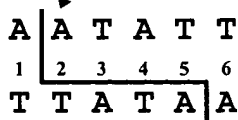


Fig. 18.19 Enzyme Editor tabbed dialog box

Enter information about the new REN as follows:

- **General Tab:** Enter the name of the REN in the Name text box.
- **Enzyme Tab:**
 - Enter the enzyme's recognition string in the Recognition String text box
 - In the Cleavage Point field on the Enzyme tab, enter the number of the nucleotide immediately after the direct-strand cleavage point. The following figure demonstrates how cleavage points of palindromic sites are defined.

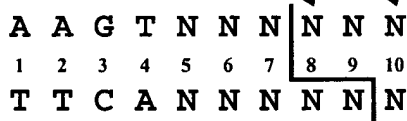
Cleavage Point = 2



If the REN has a non-palindromic recognition site, the Cleavage Point on Complementary Strand field will be enabled. In this field, enter the number of the nucleotide immediately after the complementary-strand cleavage point. The following figure demonstrates how cleavage points are defined for non-palindromic sites on both direct and complementary strands:

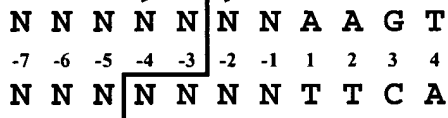
Cleavage Point on Complementary Strand = 10

Cleavage Point = 8



Cleavage Point on Complementary Strand = -4

Cleavage Point = -2



The preview box on the Enzyme tab helps you to enter cleavage points.

○ In the Description box on the Enzyme tab, enter the REN's description.

- **User Fields tab:** Add custom data in a form of fields. For details, refer to page 256.
- **Comments tab:** Enter text comments.
- **Keywords tab:** Enter key words for the REN, following the directions on page 222.

When all data is correct, choose **OK** to save the enzyme and return to the Database Explorer.

Oligo Operations

These operations are applicable to oligonucleotides only.

- **New Oligo** opens the New Oligo dialog box where you can create a new database oligo (Fig. 18.20). *The New Oligo and Edit Oligo dialog boxes are the same.*

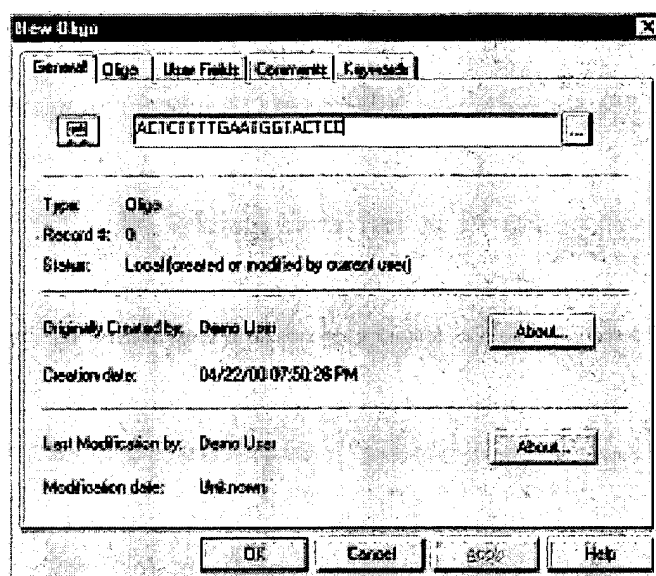


Fig. 18. 20 New Oligo dialog box

Enter information about the new (or to-be-edited) oligo as follows:

- **General Tab:** Enter the name of the oligo.
- **Oligo Tab:**
 - **Nucleotide Sequence:** Enter or edit the oligo's nucleotide sequence. Valid characters: ATUCCG.
 - **Select the oligo type.**
 - **Complementary box:** Check to replace the oligo sequence with the complementary one
 - **Description box:** Enter or edit the oligo's description.
- **User Fields Tab:** Add custom data in a form of fields. For details, refer to page 256.
- **Comments Tab:** Enter text comments.
- **Keywords Tab:** Enter keywords for the oligo. For details, see page 222.

When all data is correct, choose **OK** to save the oligo and return to the Database Explorer.

Analyze Oligo: With an oligo highlighted in the Database Objects pane, select **Analyze > Analyze Oligo** or **Analyze Oligo Duplexes** to open corresponding dialog boxes. These dialog boxes are both described in Chapter 21.

Gel Marker Operations

These operations are applicable to oligonucleotides only.

- **New Gel Marker** opens the Gel Marker Editor tabbed dialog box (Fig. 18.21) where you can enter (or edit data) for a gel marker.

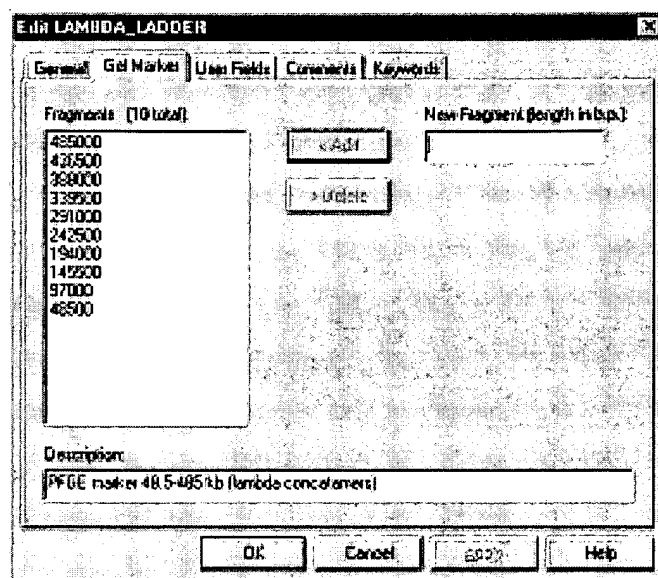


Fig. 18. 21 Gel Marker Editor tabbed dialog box

You may enter information about the new (or edited) gel marker as follows:

- **General Tab:** Enter the name of the gel marker.
- **Gel Marker Tab:**
 - **Fragments list box:** Lists by length all the fragments making up the marker. To add a fragment to the marker, enter its length in bp in the New Fragment box and press **Add**. The fragment is added to the fragment list. To remove a fragment from the marker, select it in the Fragments box and press the **Delete** button.
 - **Description box:** Enter the gel marker's description.
- **User Fields Tab:** Add custom data in a form of fields. For details, refer to page 256.

- **Comments Tab:** Enter text comments.
- **Keywords Tab:** Enter keywords for the gel marker. For details, refer to page 222

When the information is correct, choose **OK** to save the Gel Marker and return to the Database Explorer.

Drag and Drop


You can transfer objects by selecting them and using the LEFT-CLICK + DRAG technique to move them to another location. The result of drag/drop is equivalent to copy/paste operations, except that drag/drop leaves the Clipboard contents unchanged.

If you right-click + drag, when you drop, you have the option to **Copy [Here]** or **Cancel** the operation. In case of transfer between subbases of the same database, a third option, **Move Here**, allows you to combine two operations: add selected objects to another subbase and exclude them from the current subbase. *The Move operation is not available if the source subbase has its contents maintained automatically.*



Edit Menu Commands

The menu options under Edit are available for all database objects and are typical of other Windows Explorer applications. They are described as follows:

Copy/Paste

- Copy copies selected database objects and places them in subbases of the same database, local and shared databases, and databases and the file system. Selected objects, their source and names are copied to the Clipboard. You can also use the Copy button (). This mode of Copy copies meaningful biological data to be pasted into other biological analysis/management applications.

Notes about Copy:

- **Copy** using the Camera feature, by pressing the **Camera** button () , copies data as it appears on the computer screen for pasting into a word processor for, for example, preparation of publication documents.
- Because only one copy can exist on the Clipboard at one time, the Copy command replaces previous Clipboard contents.
- **Paste**, pastes copied data in one of four possible locations: (You can also use the Paste button ().

- The same subbase of the same database where they were copied. This is an alternative way to duplicate copied objects.
- Into another subbase of the same database where they were copied.
- Into another database such as a shared database. The resulting actions create a temporary archive for selected objects¹ and import it into the target database.
- To a file system or vice versa. If you paste copied objects to the Windows 95/98 or Windows NT Explorer, Vector NTI Explorer automatically creates an archive file containing copied objects². If you paste files from the Windows Explorer to Vector NTI Explorer, the files are imported into the target database. Vector NTI Explorer is usually able to recognize the types of the pasted files automatically and take appropriate actions. When it cannot determine the type of imported files, it allows you to select the type or cancel the operation.

Note: If database objects are renamed or are modified after copying to the Clipboard, when you Paste the object you may get unexpected results. Remember that the actual data are collected when you paste data, not when you copy them.

Explorer Display Options

The Explorer's database object pane can work in one of four view modes, selected under the View menu or by clicking on the View Mode button arrow, displayed at the right end of the toolbar (Fig. 18.22): Icons, Small Icons, List and Details. By default, the Database Explorer shows objects in the Details mode.

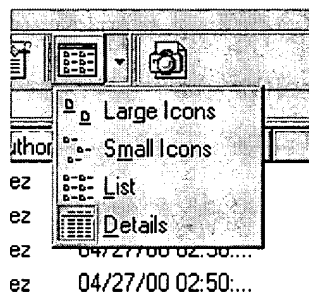


Fig. 18. 22 Display options

In the first three modes, only object icons and names are displayed. The Details view displays object icons, names and the values of object fields displayed in columns.

¹ In case the transfer of molecules, the archive may also contain parents of the selected molecules. See the Explorer Options section for details.

² For molecules, the type of the created file depends on the Explorer settings. See the Explorer Options section for details.

Options Dialog Box

To view or modify options related to the current database table display, select **View > Options** to open the Options dialog box (Fig. 18.23). *To set options for a table other than the active one, switch to that table first.* All of your settings are preserved between Vector NTI sessions.

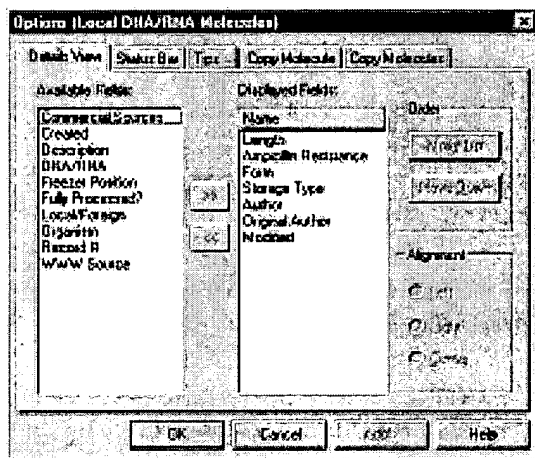


Fig. 18. 23 Options dialog box

Details View Tab

On the Details View tab, you can select the fields to be displayed (in columns) for each object type, change the order of columns and the text alignment of each column.

Currently displayed columns are listed in the Displayed Fields list box. To customize the displayed columns, in the Available Fields list box, select fields to be displayed and press the (**>>**) button. To remove fields, select them in the Displayed Fields list, and click the (**<<**) button. To modify the alignment of displayed data, select among the Alignment radio buttons. The descending order in the list is the left to right display order of the columns. To modify that, select fields and use the **Move Up** and **Move Down** buttons. Click **OK** to apply the settings.

This page can also be opened with a right click on any column heading in Database Explorer. When opened from that source, it is called the Columns page.

Status Bar Tab

This tab allows you to choose the type of information displayed on the Status Bar for a single selected object in the object pane. The default selection, **Description**, describes the item selected. If you select one of the fields, that value for the selected object is displayed. The **1 item selected** option simply describes the selection.

Tips Tab

When you pause the mouse pointer over an object in the list pane of the Explorer, a popup message (a 'tip') appears containing information about the object under the mouse pointer. On the Tips Tab, you can configure what is displayed.

Copy Molecule(s) tab (DNA/RNA or Proteins Molecules Table)

These two tabs let you select the preferred file format for a single molecule or a set of molecules export (either via copy/paste or drag/drop to the file system or other programs). Each tab includes detailed explanations of the available options.

Note: 'Parents' for proteins are 'DNA parents'.

Click **OK** to apply your settings to database display. All of the settings in the Database Options dialog box are preserved between Vector NTI sessions.

Modifying Database Object Column Widths

Column width settings can be changed directly. Move the cursor to the divider between columns where it changes to a two-headed arrow. Drag the divider right or left with the cursor until the column is the desired width.

Sorting Objects in Database Explorer

If you are in the Details View Mode, click on the column header to sort column contents. Clicking on the same column header for a second time reverses the sorting order.

To select descending or ascending sorting order for columns, or sort database objects by name or any of the displayed fields in any view mode, select **View > Arrange Icons**.

Opening Explorer Window At Startup

By default, Vector NTI opens the local Explorer window for Molecule tables at the time you start the program. To turn this feature on or off, select **Edit > Options** on the menu bar of Vector NTI's workspace window. Check or uncheck the **Open Local Explorer At Startup** box on the General tab of the Options dialog box.


Database Reports

Reports on Database Explorer's contents, suitable for word processing and spreadsheet programs, can be generated. The Database Objects Pane should contain all objects you would like to include in the report. If you do not have a subbase containing all objects you need, select them and create a new subbase or generate the report on selected objects only.

To prepare a report:

1. Select a database table and object subbase. Make sure Explorer is in the Details View mode.
2. To select the objects' fields to be displayed, right click on a column heading, select **Columns**, opening the Columns display dialog box. Move the fields between the Fields

columns, as described above in the Display View Tab section. Click **OK** to apply the settings.

3. Sort your objects by clicking on column's headers.
4. Select **Edit > Camera** or press the **Camera** button () on the Explorer's toolbar. In the Camera dialog box, select the objects to be included in the report its destination.
5. Switch to a word processor, spreadsheet or database application and paste/import the report data there.
6. Vector NTI copies the report data in one of two different formats, depending on the copy location:
 - Plain text format with the TAB character used as column separator and the newline character as a row separator (sometimes called the "CSV" format) when copying to the Clipboard.
 - Rich Text format (RTF) when copying to a file.

Explorer Shortcuts

Database Explorer offers many shortcuts to make your work more productive:

- Drag one or more molecules from the local database Explorer window onto the main Vector NTI window or into other Vector NTI Suite applications
- Drag molecules or other database objects into subbases
- Open Windows Notepad and drag/drop a molecule on its window. The molecule is displayed in the text format you chose for file system export (see Copy Molecule page in Explorer Options section).

Here is the list of useful Vector NTI Explorer keyboard shortcuts:

<i>Shortcut</i>	<i>Action</i>
F2	Rename
F3	Search
F4	Select table
F5	Refresh
F6 or TAB	Switch to another pane
Enter	Open selected object
Ctrl+Enter	Show Properties

Table 18. 2 Vector NTI Explorer keyboard shortcuts

Open/Save As Dialog Boxes

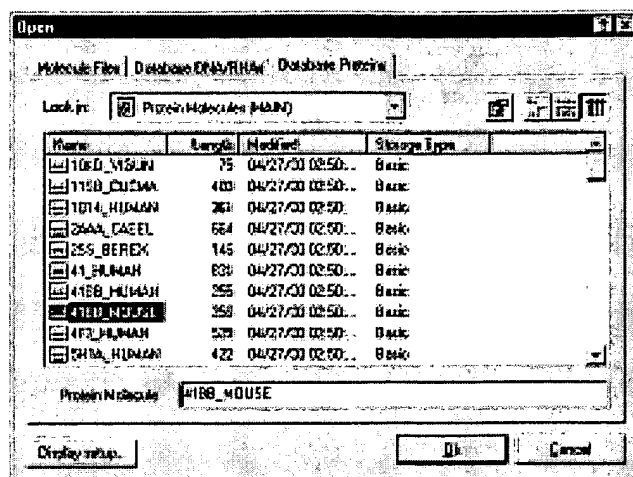



Fig. 18.24 Open and Save As dialog boxes

Many features of Vector NTI Explorer are available in the Open and Save As dialog boxes (Fig. 18.24) appearing in many situations such as when you open a Molecule Display window or save a modified molecule to the database. These dialog boxes display object lists in the same way the Explorer does. You can:

- select between Small Icons, List and Details view modes
- choose object fields to be displayed in the Details mode
- sort by any one of displayed fields
- display the Object Properties dialog box for selected objects.

Select the view modes with the buttons on the upper right or from the shortcut menu. Since there are no menu bars, select Properties (with an object selected), Options or Columns from a shortcut menu from an object or column header (in the Details view). You can also use the

Properties button (). The Columns page is managed as described under Detail View Tab on page 246.

Contact Manager

Vector NTI is increasingly used as a communication tool for exchanging information between researchers. Contact Manager is a tool for creating, updating, deleting and organizing contact records.

To launch Contact Manager, select **Database > Contact Manager**:

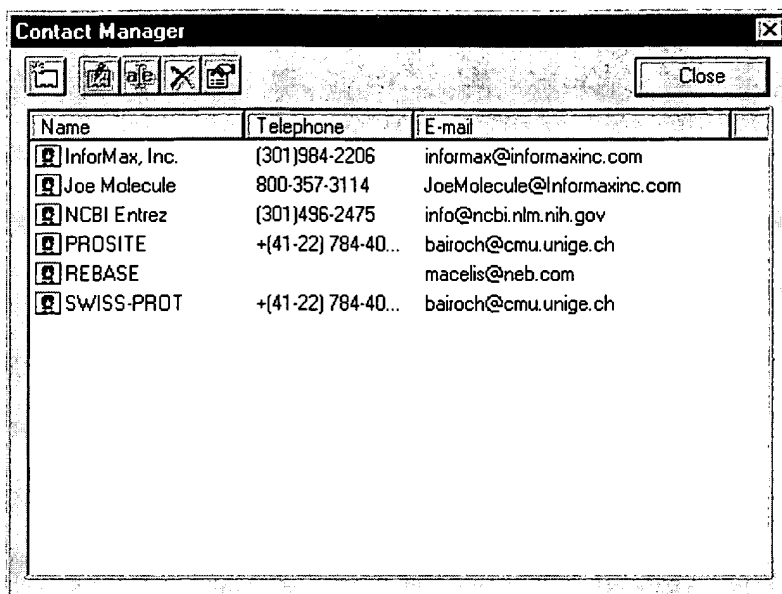


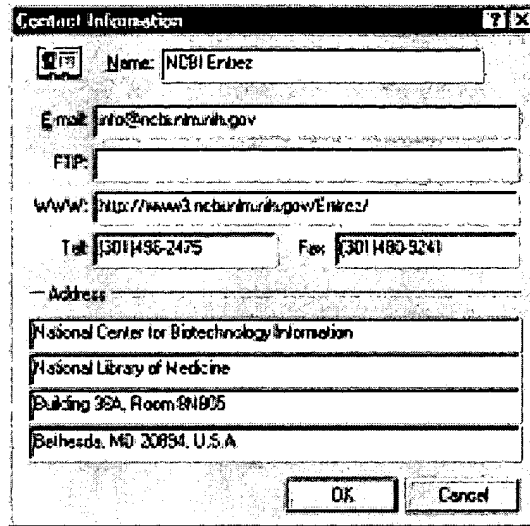


Fig. 18.25 Contact Manager dialog box

The Contact Manager dialog box displays a list of all stored contact records (Fig. 18.25). Many user interface features of the Contact Manager are similar to those of the Open/Save As dialog boxes described above. You can choose record properties to be displayed, sort by any one of the displayed properties and display the record properties dialog for selected objects. For operations on the Contact List, press one of the following buttons:

Add New or Edit Contact Records

- Press the **Add New** () or **Edit** buttons () to open the Contact Information dialog box (Fig. 18.26), where you can enter new data or edit the data for a current contact. Only the Name box is mandatory for the new contact record. All other fields may be left empty and edited later if you don't know the appropriate data.






The 'Contact Information' dialog box contains the following fields:

- Name:** NCBI Entrez
- E-mail:** info@ncbi.nlm.nih.gov
- FTP:** (empty)
- WWW:** http://www3.ncbi.nlm.nih.gov/Entrez/
- Tel:** (301)435-2475
- Fax:** (301)480-9241
- Address:**
 - National Center for Biotechnology Information
 - National Library of Medicine
 - Building 36A, Room 8N805
 - Bethesda, MD 20894, U.S.A.

Buttons: OK, Cancel

Fig. 18. 26 Contact Information dialog box

Choose **OK** to save the contact record and return to the Contact Manager.

- Delete () deletes the selected contact record from the list. Confirmation is requested before the contact record is deleted.
- Rename () changes the name of the selected contact record in an edit box.
- Properties () displays the properties of the selected contact record. Only indexed contact fields—Telephone and E-mail—are displayed in the Properties dialog box. Use the Edit operation to view/edit the entire contact record.

User Field Manager

The User Field Manager dialog box maintains a list of user field definitions necessary to provide an appropriate description of the object. Some interface features of the User Field Manager are similar to those of the Contact Manager just described. You can add User Field Definitions, select properties to be displayed, sort by displayed properties and modify User Field Definition properties.

Tip: To walk through a detailed exercise using the User Field Manager to create a new field definition and apply its values to a group of database object, see the Database Tutorial, Chapter 8.

Select **Database > User Field Manager**, opening the User Field Manager dialog box (Fig. 18.27):

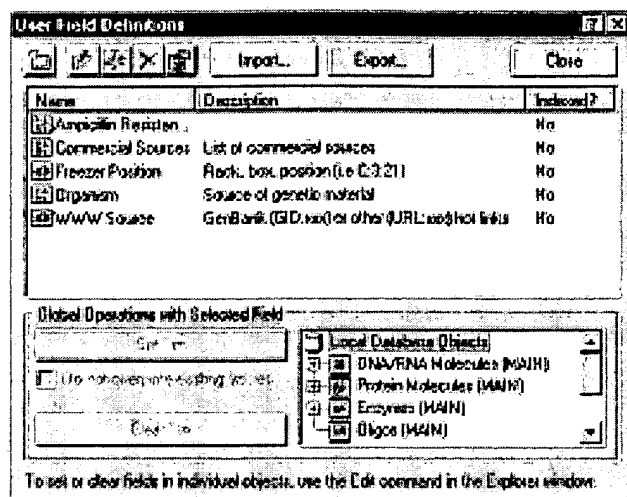




Fig. 18. 27 User Field Manager dialog box

The operation buttons in the upper left corner perform various operations, described below, on the User Field Definitions. Select the User Field Definition in the list, then press the appropriate button. *The Add New operation does not require any items to be selected.*

Note: You cannot use the name of a system field (See the System Fields Table on page 218 as the name of a new User Field you define. If you really want to name your field 'Default', you can use 'default' or 'DEFAULT.' *Vector NTI field names are case-sensitive.*)

- Add New () or Edit () (with a field selected) opens the User Field Definition dialog box (Fig. 18.28) where you can create or edit User Field Definitions.

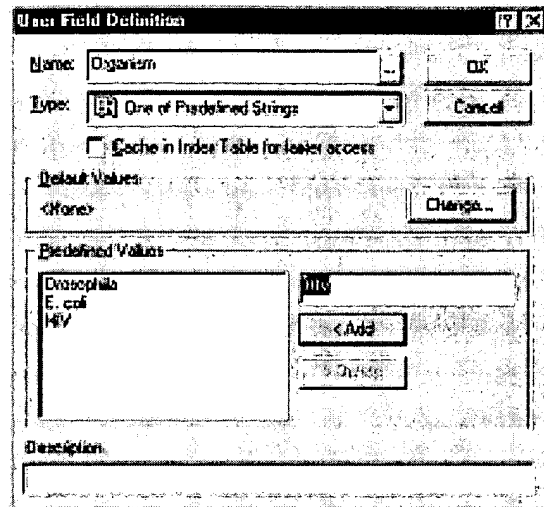


Fig. 18. 28 User Field Definition dialog box, where you can create or edit User Field Definitions

- **Name:** In the text box, enter (or edit) the name of the new user field. The user field will appear under this name in the descriptions of database objects and in the Database.
- **Type:** Select the data type that the user field will accommodate from the data type choices listed in the drop-down menu:
 - *String* - arbitrary text string, including numbers and symbols (e.g. a word, sentence or description)
 - *Yes/No* - binary field which may be set only to “Yes” or “No”
 - *List of Strings* - list of arbitrary text strings of any length
 - *One of Predefined Strings* - field which may be set only to one of predefined text strings from a user-defined list
 - *List of Predefined Strings* - field which may be set only to any subbase of predefined text strings from a user-defined list
- **Cached Fields:** Any User Field can be indexed and “cached” in the database tables. Cached fields are stored in a special memory subsystem from which they can be more quickly recalled. To cache a User Field, check **Cache in the Index Table**. Because cached fields occupy more RAM in your computer, we recommend caching only frequently used fields, for instance, those displayed in the Database Explorer in the Details mode of the Vector NTI Explorer or in the Open/Save As dialog boxes.

If Vector NTI Explorer slows down when displaying an object’s fields in the Details View mode, open User Field Manager, select the field, press the edit button and cache the field.

Because cached and ordinary fields have no functional differences, you can turn this option on or off any time you need it.

- **Default Values:** Any User Field may have a default value or values that are displayed when you attempt to fill the previously blank field of the database object. To change the default, press the **Change** button.
- **Predefined Values:** This section is enabled only for fields of the One of Predefined Strings and List of Predefined Strings types. For those fields, enter one or more value strings to choose from. These values are displayed as a list of available options when you try to fill or change the actual object's fields. Use the text box and the **Add** and **Delete** buttons to define the predefined values for the User Field.
- **Description:** Enter a description of the field to help you fill or edit the value of the field in a database object. The description entered here is displayed in the field value dialog box for this field as well as the popup label displayed whenever you pause the cursor on the field name in the User Fields tab of object editors (see below).

Note: To save space on your computer's screen and still allow you to see which predefined values are selected, you may define these values using the 'abbreviated' format: abbrev=full form as shown below (these are some of predefined values for a sample "Commercial Sources" field for enzymes):



A=Amersham Life Sciences-USB
B=Life Technologies Inc, Gibco-BRL
C=Minotech Molecular Biology Products
D=Angewandte Gentechnologie Systeme

Vector NTI displays only abbreviated form (for example A, B, D) in the Explorer windows and other places, but you will see the full forms in value-editing dialogs.


Click **OK** to return to the User Field Manager.

When you have edited data, Vector NTI automatically rebuilds the database indices.

Back in the User Field Manager:

- Rename () lets you change the name of a selected User Field. In the enabled text box, change the field name. *After renaming a User Field, Vector NTI automatically rebuilds the database indices.*
- Delete () deletes a selected User Field Definition from the Field Definitions list and, optionally, to delete field values from database objects. Confirmation is requested before deletion.
- You are also asked to confirm whether to delete the appropriate User Field values from all database objects. Deleting only the User Field Definition does not affect consistency of the database information. When you delete the Field Definition from the list, Vector

NTI no longer prompts you to fill this field in new objects and existing objects where it was blank. However, unless you delete the field value from database objects, Vector NTI still stores and displays the existing field data in the database objects. If you try to edit the existing value of that “unlisted” field in any database object, Vector NTI will ask you to add the definition of the field back to the User Field Definition List before proceeding.

- Properties () display the properties of the selected User Field Description. These properties can be also displayed in the User Field Manager dialog box by opening the shortcut menu from the list headings and selecting Columns.

Import/Export

To simplify the maintenance and sharing of User Fields across several databases and/or several computers, you can save your User Field Definitions as “archives.”

To export User Field Definitions to the archives, press the **Export** button. In the dialog box that opens, select the definitions to be exported and press **OK**. In the standard File Save dialog box, locate the directory and archive file name for storing the exported definitions. Press **OK** to create the archive.

To import User Field Definitions from the archive, press the **Import** button. In the dialog box, locate the directory and archive file to be read. Press the **OK** button, initiating the import. After importing, Vector NTI automatically rebuilds the database indices.

Set In/Clear In

In the User Field Manager, you can assign or “set” a value or clear all values for a particular User Field in a group of database objects.

- To set a value for a user field:

Select the user field in Field Definition List (large window). In the database tree in the lower right corner, select a subbase of database objects that the field would pertain to. You may choose all database objects regardless of their type or a particular subbase for database objects of a particular type (molecules, enzymes, oligos or gel markers). If you don’t want to overwrite existing values of the User Field in the selected group of objects, check the **Do not overwrite...** box.

In the Global Operations section, press the **Set In <user field >** button. In the dialog box that opens, specify the field value string to be set and press **OK**. Vector NTI shows a description of the action about to be performed, asks for your confirmation, then inserts the specified value into the appropriate objects in the subbase you selected.

- To clear all values of a user field:


Select the user field. Choose a subbase of database objects in the database tree. You can choose all database objects regardless of their type or a particular subbase for database objects of a particular type (molecules, enzymes, etc.)

Press the **Clear In <user field >** button. Vector NTI warns you of the action about to be performed. If you confirm the operation, it clears the values of the specified user field in the specified objects.

To set or clear fields in a set of individual objects not currently represented by an existing subbase, close the User Field Manager, create the required subbase in Database Explorer and return to the User Field Manager. Alternatively, you may change field values in individual objects by editing them in the Explorer one by one as described below.

Editing User Fields in Individual Objects

When a User Field is defined in the User Field Manager, you can edit it for any database object using the User Fields tab in the Edit <object name > dialog box (Fig. 18.29). To open the Edit dialog box from Database Explorer, select the object in the Object Pane and press

the **Edit** button () or select **Edit > <Object Name >** or the corresponding command from the shortcut menu:

User Fields Tab

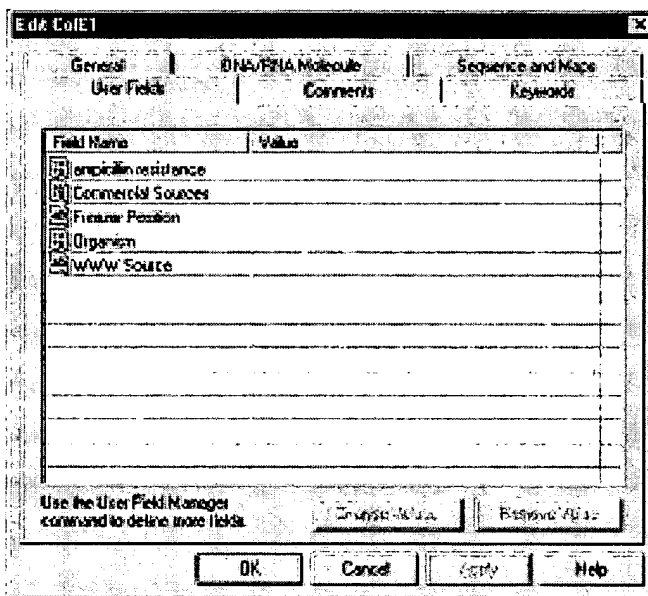



Fig. 18. 29 Edit <object name > dialog box

All of the tabs of this dialog box except the User Fields tab are discussed in the section, Editing Database Objects in this chapter. User Fields shows a list of all User Fields currently applied to the object being edited and the list of blank fields defined in the local User Field Definition list but not yet specified for the current object.

To set or edit any field, double-click the field name or select the field name and press the **Change Value** button. If the field you want to edit is not listed in the local User Field Definition list, enter the field definition first. When added, the appropriate field value dialog box is displayed. To quickly clear any User Field of the object, select the name of the field and press the **Remove Value** button.

User Fields in Molecule Display Windows

You can view and edit User Fields tab from Molecule Display windows. To open the Edit dialog box, select **Edit > <object> Properties** or in the Text Pane, select Molecule Properties on the shortcut menu opened from the topmost folder <Object Name> or click on the  box to the left of the <Object Name> folder.

Cut, Copy and Paste

To Cut, Copy, or Delete a region of the molecule's sequence, select the region and choose the appropriate command from the Edit menu. *If a region to be deleted overlaps with features, a confirmation dialog box opens, allowing you to keep or remove affected features.* All the features inside the deleted region will be removed without confirmation; positions of other features will be adjusted accordingly.


A copied sequence is placed on the Clipboard in the standard text format, so you can freely exchange the sequence data between Vector NTI and other applications. All sequence formats recognized by Vector NTI can be pasted from the Clipboard. Sequence formats are described in detail in Chapter 15 (Import section).

To paste a sequence from the clipboard, set the cursor to the insert position. Choose **Edit > Paste Sequence**, opening and displaying the sequence in the Insert Sequence dialog box. Press the **OK** button, and the sequence is inserted at the cursor position. If the insertion point is within a feature, a confirmation dialog box opens, to let you keep or remove the affected features. The positions of all features surrounding the insertion point are updated automatically.

Insert a new sequence fragment manually by setting the cursor at the insertion point; choose **Edit > New > Insert Sequence**. In the Insert Sequence dialog box, enter the sequence to be inserted. For more information on editing a sequence, see Chapter 20.

Finding Protein Sequence Fragments

To quickly find a defined fragment on a sequence, in the Sequence (or Graphics) Pane, press

the **Find** button , select **Edit > Find Sequence** or press CTRL + F. In the Find Sequence dialog box (Fig. 19.17), enter the desired sequence and set the acceptable mismatch tolerance. The sequence can contain any standard IUPAC symbols for amino acid sequences. (See Appendix C.)

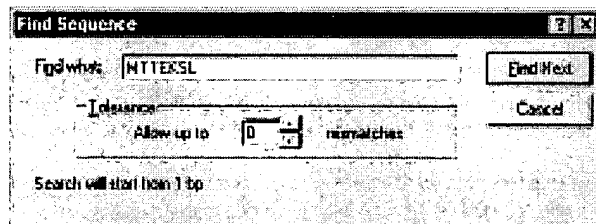


Fig. 19. 17 Find Sequence dialog box

Press the **Find Next** button. The search begins from the current caret position or the current selection. A located fragment is automatically selected in the Sequence Pane. If none are found, the caret does not move from its current position.

Chapter 20 Editing Molecule Content

Introduction

DNA/RNA and protein molecules can be edited in two environments:

- A Molecule Display window Graphics Pane, allowing editing of the molecule content in the graphics-oriented environment.
 - Molecule Display windows give you immediate visual feedback and more automatic processing (that is, automatic adjustment of functional signal coordinates on sequence insertions and deletions).
- Molecule Editor, a text-based dialog box opened from a Molecule Display window of either molecule type.
 - Editing a molecule in the Molecule Editor is more convenient if you want, for example, to delete ten functional signals from the functional map.
 - Molecule Editor is the only way to create a molecule from scratch. (*Molecule Display windows cannot show molecules of zero length*).


Edit Operations in a Molecule Display Window

Molecule Editing

Vector NTI has two modes of editing operations in Graphics Panes.



- Molecule Editing mode (default) in which the Graphics Pane serves as a visual environment for viewing and editing a molecule map and sequence and for selecting DNA molecule fragments for construction and design. *In Molecule Editing mode, the molecule itself can be modified. Molecule Editing mode is the subject of this chapter.*
- Picture Editing mode where the *graphical display* of an image such as the format and arrangement of individual graphics objects is edited. This mode is useful for preparing publication-quality figures of the molecule. Picture Editing mode is discussed in Chapter 19.

A Molecule Display window opens in Molecule Editing mode by default. Molecule Editing mode is used for viewing and editing graphical maps of a molecule, viewing ORFs and motifs (for DNA molecules), and editing molecule's sequence. This mode is also used to select DNA molecule fragments for construction and design.

If the display window is in Picture Editing mode, the **Edit Picture** button () is shown in depressed state and **View > Edit Picture** is checked.

Selection Techniques on the Graphical Map

The following techniques can be used for making selections in Molecule Editing mode:

- On the Graphics Map, point the cursor at a symbol or label. If pointing at a restriction site or motif, the cursor becomes a crosshair with a word “site” (). With the cursor paused at the site, a pop-up label appears, giving the type and site position. If pointing at a functional feature or an ORF, the cursor becomes a hand () and upon pausing, it displays a message telling you the type, name, and location of the feature or ORF. Click on the symbol, site or its label to select it.
- Choose **Edit > Set Selection**. In the Set Selection dialog box, enter the coordinates of the region to be selected. Both start and end nucleotides are included in the selection, so to select the nucleotides or amino acids 50 through 60 you should enter From: **50**, To: **60**. Press **OK**. The coordinates of the selection are shown in the Selection Box on the status bar at the bottom of the screen.
- Click on the Set Selection box on the Status bar, opening the Set Selection dialog box. Enter the region coordinates in the Set Selection box. Click **OK**.

A selected region is marked with a selection wireframe on the Graphics Pane (Fig. 20.1). Concurrently the selection is highlighted in the Sequence Pane.

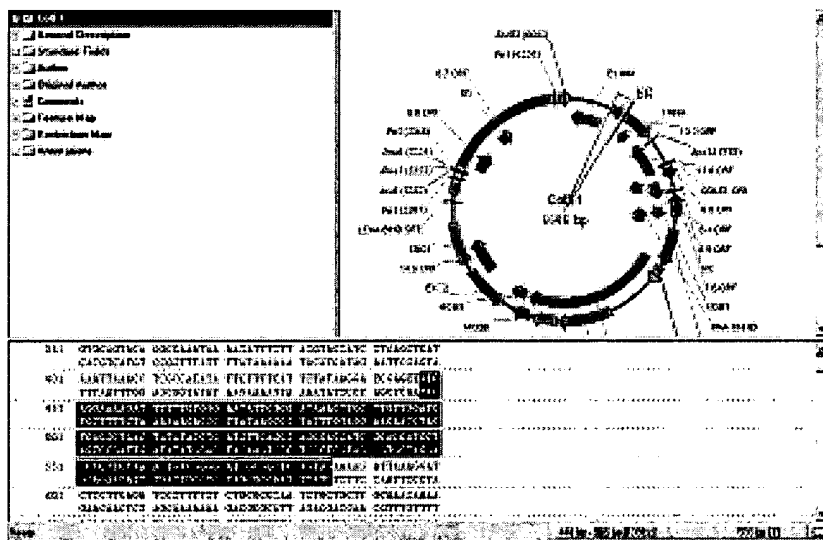



Fig. 20. 1 Wireframe selection on the Graphics Pane

A caret (heavy black marker) appears at the “active” end on the wireframe and the sequence at either the 5’ or 3’ end (a or c end for proteins). *The caret position is also shown in the caret position box on the status bar.*

The caret makes it easy to modify the ends of the selection in the Graphics Pane:

- To activate an end of the selection, click on it with the mouse or click on the HOME or END key.
- Hold the cursor over the active end; when it changes shape to the cross-hair with the 5' ( (or 3', or a/c mark), click the mouse button and drag the end to the desired position (watch the selection box on the status bar while you are dragging it). Drag the 3' end of the selection in the same way.
- To move the active end of the selection one residue at a time, hold the SHIFT + LEFT or RIGHT ARROW key to move the active end of selection one nucleotide/amino acid at a time.
- To move the caret 10 residues at a time, hold down the CTRL key (do not release the SHIFT key) with arrow keys. When the CTRL + SHIFT + LEFT or RIGHT ARROW key.

Note: If you release your hold on the ctrl + shift keys, you will lose your current selection.

Setting The Caret Position On The Graphical Map

To set the caret to a position of the molecule, choose **Edit > Set Caret Position** or click on the Set Caret box on the Status Bar. In the Set Caret Position dialog box, enter the coordinate of the nucleotide/amino acid *following* the caret. The coordinates start from 1; so to set the caret before the first nucleotide of the molecule you should enter 1. Press **OK**. The position of the caret appears on the Graphics and Sequence Panes and in the caret position box on the status bar.


Note: Setting a coordinate in a Set Selection or Set Caret Position dialog box replaces any previous selection.

Selection Techniques for Choosing DNA Fragments for Recombination

The simplest way to select DNA fragments for recombination is to use the Fragment Wizard, discussed in detail in Chapters 22 and 23. However, if you wish, you may select regions as described above.

To select more than one functional signal at a time, click on each while holding down the SHIFT key. The wireframe will include all selected signals (plus intervening nucleotides). Press the TAB key to move the selection ends to the next gap between selected functional signals; press SHIFT + TAB moves the selection ends to the previous gap between selected functional signals. *If you prefer to select features without intervening nucleotides, select the fragments one by one and add them to the Goal List individually.*

To set a 3' or 5' end of the selection to a restriction site, press HOME or END to move the caret to the desired end of the selection and hold down the SHIFT key while clicking on the label of the restriction site. If an end of the selection zone is set to a restriction site, an @ symbol appears in front of that end's coordinate in the status bar.

After the fragment is defined, click on the **Add Fragment to Goal List** button () , identify the role in construction or design the fragment will play, and press **Finish**.

Editing The Molecule Map

The elements of a molecule map can be divided into two categories.

1. Items explicitly described in the molecule data (features on DNA/RNA and protein molecules and restriction sites on un-sequenced regions of a DNA molecule). These can be edited.
2. Items calculated automatically from the DNA molecule's nucleotide sequence (ORFs, motifs, and restriction sites on sequenced regions of the molecule) each time the molecule is displayed. These are not stored with the molecule; these cannot be edited.

Editing operations are as follows:

- **To edit a feature of the graphics map**, double-click on it or select it and choose **Edit > Properties**, select **Properties** from the shortcut menu or press ALT + ENTER. The Properties dialog box opens, where you can edit the properties of the selected object. *The Properties dialog box for automatic objects does not contain editable fields.*
- **To add a feature** to a molecule, select the corresponding region of the molecule and choose **Edit > New > Add Feature To FMap**. In the Molecule Feature dialog box, you can describe the new feature.
- **To add an ORF** to a DNA molecule's Feature map, select the ORF you want to add and choose **Edit > New > Add ORF to FMap**. In the Molecule Feature dialog box, you can name the feature (all fields are initialized with the information taken from the selected ORF).
- **To add restriction sites** to unsequenced regions of a DNA molecule, move the caret to the desired position and choose **Edit > New > Add REN Site to RMap**. In the Restriction Sites dialog box, the Site Positions field shows the current caret position. You can enter more than one site position separated by space. Select the name of the enzyme and press **OK**. Vector NTI adds the sites modifying the nucleotide sequence accordingly.
- **To delete an element** from the molecule map, select the element. Choose **Edit > Delete**, select the **Delete** from the shortcut menu or press DELETE on the keyboard. To remove "automatic" objects from the graphics map display, you must change the parameters in Display Setup.


Notes about molecule editing:

- If you have chosen an "automatic" feature to delete, a window appears reminding you that this feature cannot be deleted.
- You can rescind all molecule content edits by selecting **Molecule > Revert to Saved**.

Editing the Sequence from the Sequence Pane

Sequence editing operations in the Sequence Pane are similar to those in the Graphics Pane. Three editing operations make use of the standard Clipboard (Cut, Copy, and Paste) operations. Others allow you to insert literal fragments or modify existing fragments.

The copied sequence on the Clipboard is in the standard text format, for free exchange of the sequence data between Vector NTI and other applications.

To Paste (“insert”) a cut or copied sequence, set the cursor, which in the Sequence Pane becomes a vertical I-beam (), to the insert position. Paste with a toolbar button or select **Edit > Paste Sequence**.

To insert a new sequence fragment manually, click the I-beam at the insertion position; choose the **Edit > New > Insert Sequence**. In the Insert Sequence dialog box, enter the sequence to be inserted. Click **OK**. If the inserted sequence will affect a feature, you are given the option to delete the feature (**Delete** or **Delete All**) or keep it (**Keep** or **Keep All**) in its modified form.

Once the sequence is inserted, you can delete it manually or select **Molecule > Revert** to Molecule in Database to return to the unedited version.


Edit Operations in Molecule Editor

To open the Molecule Editor dialog box:

From Molecule Display window:

- **Molecule > Create New > Using Sequence Editor (DNA/RNA)** or **> Using Sequence Editor (Protein)** opens Molecule Editor (Fig. 20.2) for a new molecule
- **Edit > <Molecule> Properties** opens Molecule Editor for the open molecule

From Database Explorer:

- Select the DNA/RNA or Protein subbase in the drop-down menu, select a molecule in Objects list and click on the **Edit** button () or **<Molecule type >> Edit** on the Menu Bar.

The figure shows the DNA/RNA Molecule tab of the Molecule Editor opened from Database Explorer (Figure 22.2):

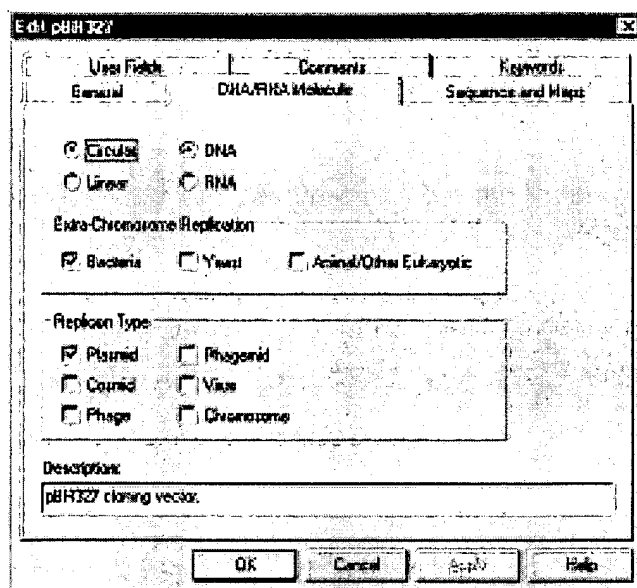


Fig. 20. 2 DNA/RNA Molecule tab of the Molecule Editor

The Molecule Editor contains information for one molecule at a time. Note these points about the Molecule Editor:

- Most tabs for protein or DNA/RNA are basically the same. Any variations are mentioned below.
- Molecule Editor opened from Database Explorer has one additional tab for sequence and feature editing. *Sequence editing of an open molecule must be performed in the Sequence Pane of the Molecule Display window itself.*
- To be edited with the Molecule Editor, a molecule must be fully processed. For instance, when you are trying to construct a DNA molecule from incompatible component fragments, Vector NTI saves the entered molecule's data but does not process the molecule completely. It allows you to load molecule data from the database into the Construct/Design Molecule dialog box, edit its component fragments, and reconstruct the molecule. But you cannot load a molecule into the Molecule Editor until it's fully processed.
- The Molecule Editor can be used to describe a new molecule from scratch. The Molecule Editor is almost identical for New Molecule (creating a new molecule from scratch) or for Edit Molecule. The New Molecule mode lets you enter all data including a sequence.

Note: You must enter a new molecule's nucleotide sequence using the Sequence Editor (Fig. 20.6) to be able to save the new molecule. If the new DNA molecule is unsequenced, it

is easier to construct it first from one DUMMY fragment using Construct/Design Molecule dialog box, and then enter its functional and restriction maps loading the molecule from the database into the Molecule Editor.

- If you have made any changes to a molecule in the Molecule Editor, you must press the Molecule Editor's **OK** button to enter the new information into the database.

Note: When a molecule is created during the design or construction process, its nucleotide sequence and functional map are generated automatically from its parents. You may add new functional signals to the map or delete or edit automatically-generated functional signals or edit an automatically generated nucleotide sequence. In that case, the system disconnects the molecule from its parents to avoid data inconsistency. The system prompts you if such a situation occurs.

Each of the tabs of DNA/RNA and protein Molecule Editors are discussed below.

The General Information Tab

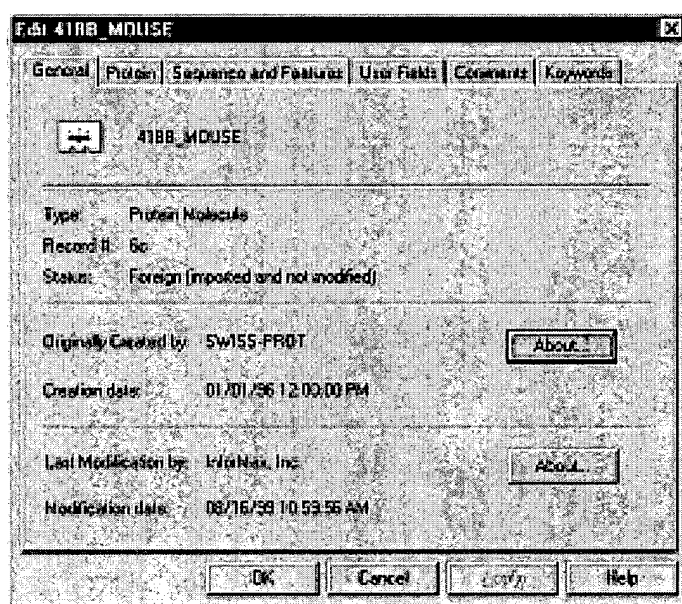


Fig. 20. 3 The General Information tab displays current status of the database object and other general molecule data

The General Information tab has the same format for all database objects (Fig. 20.3). On this tab, enter or edit a name for the new molecule. *There is no text box for entering the name, but moving the cursor close to the molecule name changes the pointer to an I-beam, enabling text entry.*

The DNA/RNA Molecule Tab

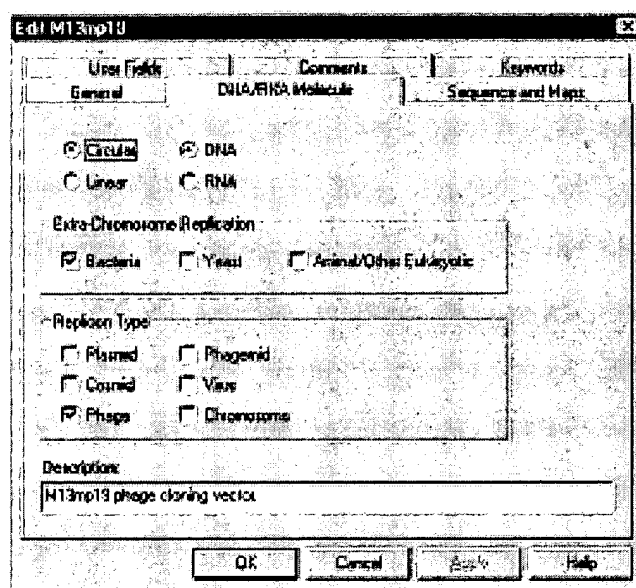


Fig. 20. 4 DNA/RNA Molecule tab

- **Molecule type:** Circular/linear and DNA/RNA
- **Extra-Chromosome Replication:** Transformation systems in which the molecule is capable of extra-chromosome replication.
- **Replicon Type:** The source of the replication origin for the molecule
- **Description:** single-line description of the molecule.

The information in these fields appears in the General Description folder when the molecule is loaded into a Display window.

The **Protein tab** contains a one-line molecule description.

The Sequence and Maps Tab (DNA/RNA)

This tab appears only when the Edit <molecule> dialog box is opened from Database Explorer (Fig. 20.5).

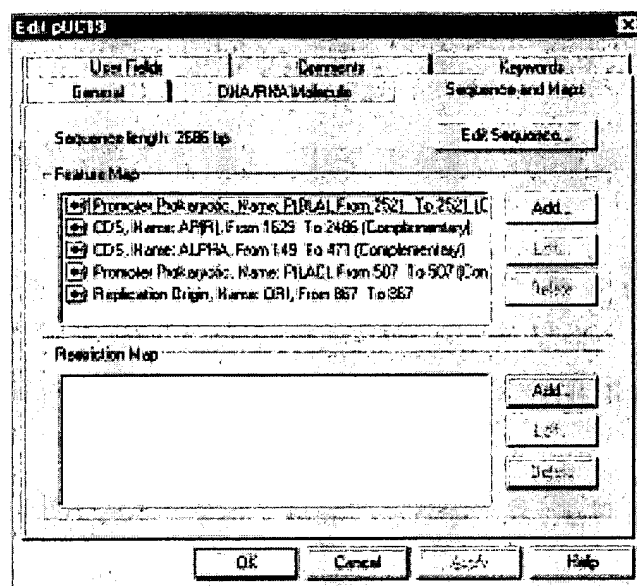


Fig. 20. 5 Sequence and Maps Tab (DNA/RNA)

Editing Sequence

From the Sequence and Maps tab, click the **Edit Sequence** button to open the Sequence Editor to edit the sequence of the molecule (Fig. 20.6).

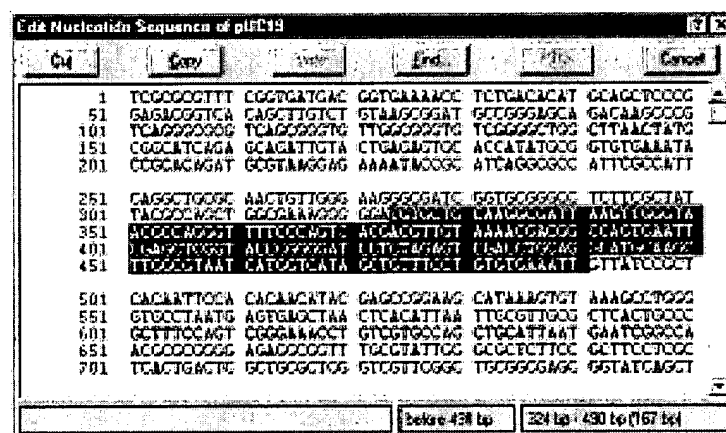


Fig. 20. 6 Using the Sequence Editor to edit the sequence of the molecule

Edit the sequence using standard text editing techniques, Cut, Copy, and Paste through the Clipboard.

Press the **Find** button to specify a string to find in the sequence and the search direction (up or down). Click **Find Next** to launch the search and **Cancel** to leave the Find dialog box.

Press the **OK** button to save any changes you made in the sequence and to return to the Sequence and Maps tab of the Molecule Editor.

Editing the Feature Map

To add an item to a molecule's Feature map, press the **Add** button in the Feature map pane of the Sequence and Maps tab. (This dialog box can also be accessed by: Select **Edit > New > Add Feature to Fmap** or right-click on a feature in the Graphics Pane. Select **Feature Properties** from the shortcut menu.) The Molecule Feature dialog box appears (Fig. 20.7):

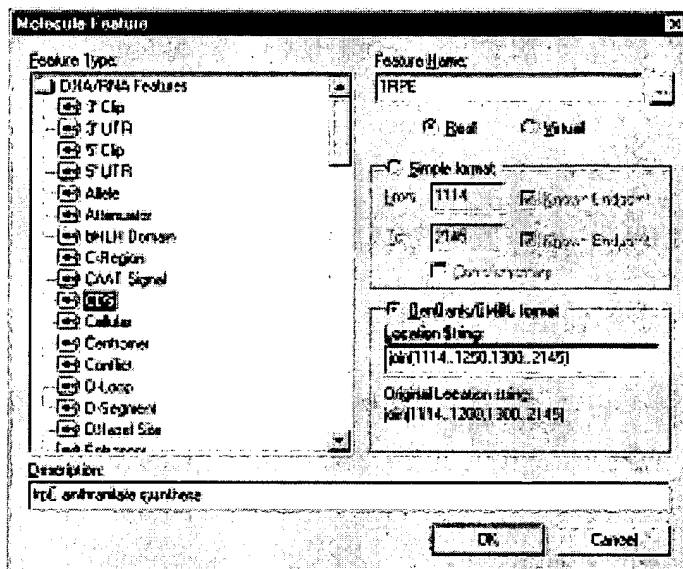


Fig. 20. 7 Molecule Feature dialog box

To add a feature, select a particular Feature Type in the classification tree on the left. Enter the specific name of the feature in the Feature Name field. To see a list of specific names for

each feature type, press the **Browse** button (....) next to the Feature Name field, opening the Feature Name dialog box, which lists the specific names available for that feature type (Fig. 20.8):

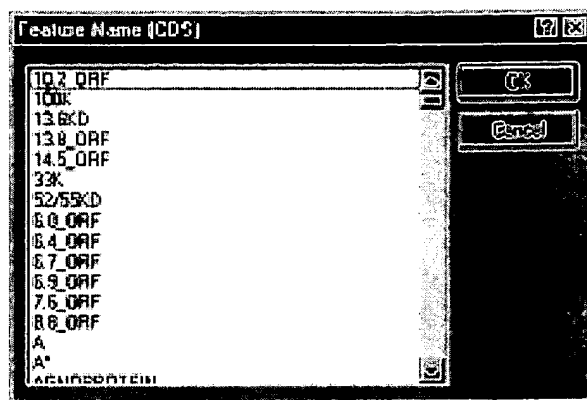


Fig. 20. 8 Feature Name dialog box, which lists the specific names available for that feature type

After selecting a feature signal name, press **OK**.

Back in the Molecule Feature dialog box, select feature positions by using one of two formats:

- 1) **Simple:** In the **From** and **To** fields, enter the starting and ending coordinates of the functional signal you are adding.

Check **Complementary** if the signal is located on the complementary strand.

Note: Vector NTI uses the currently accepted convention for the coordinates of complementary features. All coordinates are given as if on the direct strand, from the leftmost to the rightmost nucleotide of a signal, as shown in Fig. 20.9:

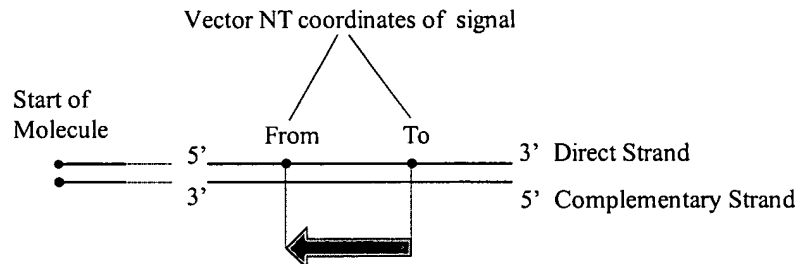


Fig. 20. 9 Defining feature position coordinates

- 2) **GenBank/EMBL format:** Set a complex location string that describes the positions of the exons and location of the features. *The full description of location string format can be found in the GenBank or EMBL sources.* For your convenience, you can always see the original location string of the feature.

In the Description text box, enter notes about the feature you are adding.

Press **OK** to add your feature and return to the Sequence and Maps tab.

To edit a feature: Double-click or highlight the feature in the Feature map pane and press **Edit**. The Molecule Feature dialog box (Fig. 20.7) opens loaded with the feature you selected. Make any changes you want, and press **OK** to apply your changes or press **Cancel**.

To remove a feature from a molecule's Feature map: Highlight the feature in the Feature map pane and press **Delete**.

Editing the Restriction Map

To add a restriction site to the molecule's restriction map: Press the **Add** button in the Restriction Map group of the Sequence and Maps tab (Fig. 20.5), opening the Restriction Sites dialog box (Fig. 20.10): (You can also access this dialog box by selecting **Edit > New > Add REN Site to Rmap**.)

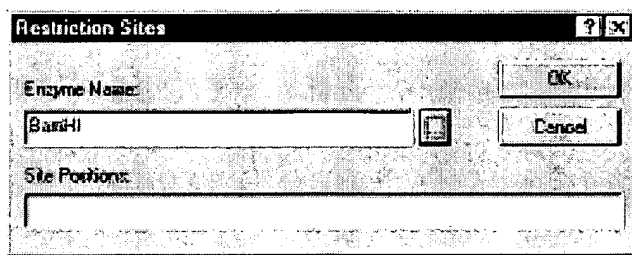


Fig. 20. 10 Restriction Sites dialog box

In the Enzyme Name field, enter the name of the restriction endonuclease or press the

Browse button () to see a list of RENs in the Vector NTI database.

In the Site Positions field, enter the nucleotide position numbers where the sites of the enzyme you have chosen are found, separated by spaces.

To edit a restriction site: Double-click it or highlight the item you want to edit and press **Edit**. The Restriction Sites dialog box appears, loaded with the site you selected. Make any changes you want, and press **OK** to register your changes or the **Cancel**. Vector NTI returns you to the Molecule Editor.

To remove an item from the restriction map: Highlight the item and press the **Delete** button in the Restriction Map group. *Sometimes Vector NTI does not allow removal of a restriction site if it is not entered manually.*

Sequence and Features Tab (Protein)

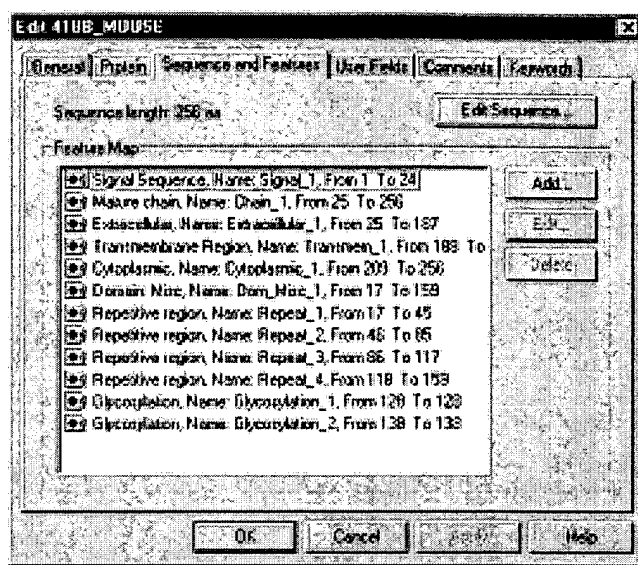


Fig. 20. 11 Sequence and Features tab (Protein)

This tab's functionality is the same as that of the Sequence and Maps tab of the DNA/RNA Molecule Editor, however, only sequence and Feature map editing are supported.

The User Fields Tab

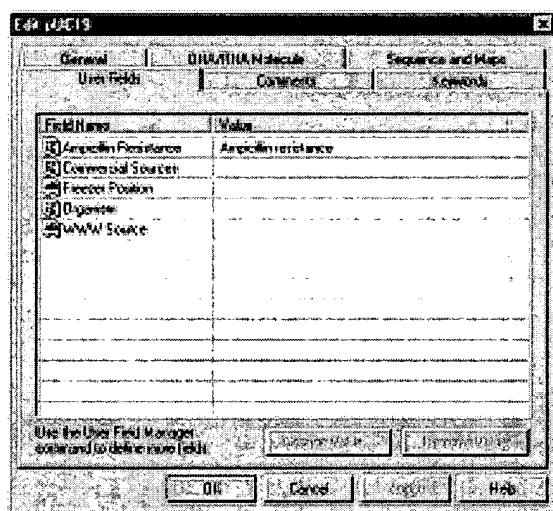


Fig. 20. 12 User Fields tab

The User Fields tab (Fig. 20.12), used for adding custom data for a molecule in the form of fields, has the same format for all database molecules. It contains a list of all User Fields currently applied to the object being edited as well as the list of blank fields defined in the local User Field Definition list but are not yet applied to that object.

- To fill or edit any field, double-click on the field name or select the field name and press the **Change Value** button. If the field you want to edit is not listed in the local User Field Definition list, Vector NTI asks you to enter the field's definition first. When the field definition is added to the local definition list, Vector NTI displays the appropriate field value dialog box.
- To clear any User Field for the object, select the name of the field and press the **Remove Value** button.
- Refer to Chapter 18 for more user field details.

The Comments Tab

The Comments tab (Fig. 20.13) has the same format for all database objects. Enter text comments of unlimited length about the molecule.

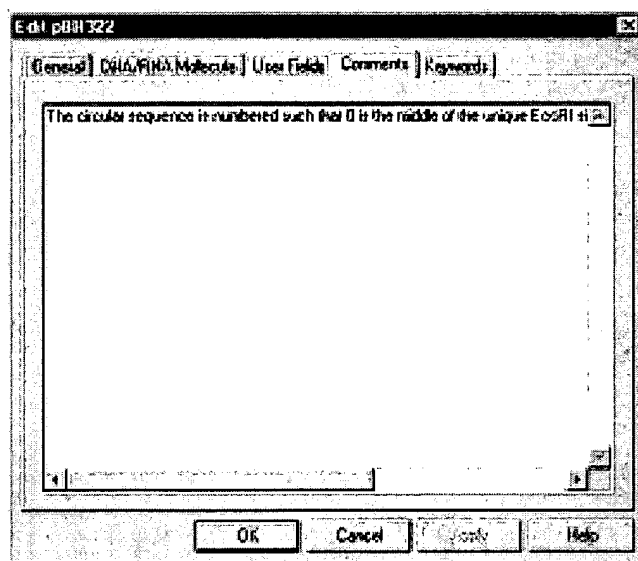


Fig. 20. 13 Comments tab

The Keywords Tab

The Keywords tab (Fig. 20.14) has the same format for all database objects. This tab lets you enter keywords useful for database search.

To add a keyword for the molecule, type a new word or select an item in the list of existing keywords. Press the **Add** button to move the keyword into the keyword list.

To remove an item from the keyword list, select item(s) press the **Remove** button.

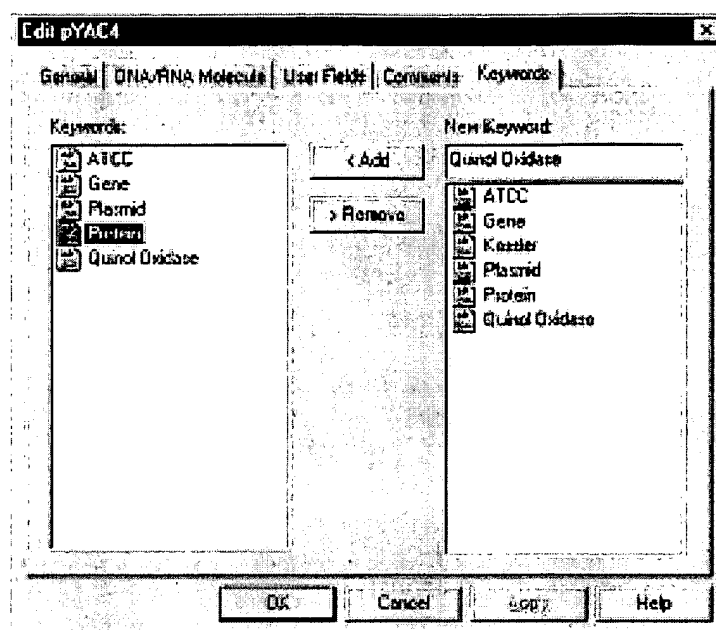


Fig. 20. 14 Keywords tab

Chapter 21 Oligo, Primers and Other Search and Analyses

Introduction

This chapter describes Vector NTI's oligonucleotide analysis functions: the design, execution and analysis results for PCR primers, sequencing primers, and hybridization probes. The chapter also covers "silent mutation" analysis, ORFs and motif searches, calculation of restriction fragments, back translating and broadcasting selections.

A major focus of molecule search and analysis in Vector NTI revolves around working with primers, oligos and probes. Because of the numbers of various dialog boxes that open as you proceed through the design and analysis of these short sequences, you may want to review the following table of primer, oligo and probe dialog boxes that summarizes the features and purposes of each.

<i>Dialog Box</i>	<i>Purpose</i>
Find PCR Primers	Specify limits for PCR primer search such as length of target sequence, output options, attach restriction sites, etc.
Amplify Selection	Similar to Find PCR Primers except that primer hybridization domains upstream and downstream from the target sequence can be specified. Primers will be generated anywhere within the designated upstream and downstream domains.
Amplify Features	Similar to Find PCR Primers except that multiple features of a specific type can be selected for amplification. Use this dialog box when you need to amplify multiple features from a single molecule.
Sequencing primers	Set parameters for sequencing and primer regions and primer; analyze primers.
Similarity of <technique> primers	Determine relationship of complementarity between primers and target sequence. Therefore, the number of nucleotides on 3' end required to have 100% complementarity with target.
Primer/Oligo Parameters	Set acceptable limits for primer/probe interaction with target sequence. Also relates to formation of secondary structures between the oligos themselves.

<i>Dialog Box</i>	<i>Purpose</i>
Primer/Oligo Quality Specifics	Assigns "importance factors" to different primer parameters. Importance factors are integers between 1 to 10, all relating to parameters specified in other dialog boxes, such as %GC, T _m , and so forth. These parameters determine how much weight should be given to each of the named specifications. For minimal importance, enter 1; maximal, enter 10.
Hybridization Probes	Set parameters for target region, output options; analyze probes.
Oligo List	Lists all oligos in the oligo database. From this dialog box, add new, edit, or analyze oligos.
Edit/New Oligo	Enter new or edit oligo data, including name, sequence, user fields, comments, keywords.
Oligo Analysis	Set limits for annealing conditions with target sequence; analyze oligo for meeting specified criteria.
Dimers and Hairpin Loops	Examine oligo's dimer and hairpin loop structures.
Oligo Duplexes	Generate all possible duplexes of selected oligos.

Table 21. 1 Primer, oligo and probe dialog boxes

PCR Primers

Vector NTI locates primers for performing PCR analysis on a DNA molecule fragment selected in the Graphics Pane of a Molecule Display window. (Vector NTI can also evaluate user-defined primers.) After selecting the target sequence for primer design, the maximum and minimum product length and primer parameters are determined. Vector NTI evaluates, rates and sorts several primer options. You can further fine-tune the primers and annealing parameters if you wish, generate and display a PCR product in a Molecule Display window, save the primers (or product) in the database, or use the product in recombinant cloning strategies.

Vector NTI provides three different modes of PCR amplification: **Find PCR Primers**, **Amplify Selection** and **Amplify Features**. The Find PCR Primers mode allows you to find primers for a DNA selection within the selected region. The Amplify Selection mode allows you to define allowed regions upstream and downstream of the selected area for primer design. The Amplify Features mode allows you to amplify one or more selected features in a molecule.

This section covers preparation for PCR analysis; the Find PCR Primers, Amplify Selection and Amplify Feature dialog boxes; the results of PCR analysis presented in PCR Analysis folders; and operations with PCR primers and PCR products.

Open a Display window for the DNA molecule selected for PCR analysis. Select the target region of the molecule and choose either **Analyze > Find PCR Primers**, **Analyze > Amplify Selection** or **Analyze > Amplify Feature**, depending on the type of PCR analysis you want to perform. (These commands are disabled if no target is defined.) This opens the corresponding PCR Analysis dialog box (Fig. 21.1, 21.10 and 21.11).

Find PCR Primers Dialog Box

The Find Primers dialog box (Fig. 21.1) allows you to search for convenient sense and antisense primers according to your specifications and to check primers' uniqueness. Pressing the **More>>** button on the **Primer** tab enables complete view of the Find Primers dialog box.

Notice the **Load** and **Save** buttons on the lower left of each tab in the Find Primers dialog box. These buttons allow you to save your PCR settings to a file and load the settings file in for subsequent analyses. This precludes having to reset the various parameters manually for frequently used PCR condition settings. You need only save/load the settings in one of the tabs and the parameters for all the tabs in the Find Primers dialog box will be saved/loaded.

Fig. 21.1 Find Primers dialog box allows you to search for convenient sense and antisense primers

Primer Tab

The options in the **Primer** tab allow you to set parameters for PCR primer search and define primers and/or primer attachments, if desired:

Primer Tab Parameters – Find Primers Dialog Box	
Region of Analysis	Enter the start and end coordinates of molecule's target region.
Product Length	Enter the maximum and minimum lengths of the molecule target region. Note: Unless you specify differently here, the minimum amplicon length may be less than the target sequence you selected.
Maximum Number of Output Options	Enter the number of sense-antisense primer pairs to be found. The actual result may contain fewer than this number if there are not enough possible primers.
Salt Concentration	Enter the PCR reaction salt concentration in mMol, if known.
Probe Concentration	Enter the value of probe concentration in pMol, if known.
dG Temperature	Enter the temperature in degrees Celsius to be used for calculating free energy values.
T _m	Enter limits in degrees Celsius for primer melting temperature (T _m) (temperature at which 50% of primer is a duplex) and the difference between T _m for sense and antisense primers.
%GC	Enter the limits of G/C percentage in the primer and the difference between GC percentages for sense and antisense primers.
Length	Enter primer length limits. Note: Nucleotide sequences such as RENs attached to a primer's 5' end are included when calculating primer length.
DNA/RNA radio button	Select the type of target nucleotide sequence.
More>> and Less<< buttons	Extend or contract the lower portion of the Primers tab, which contains the user-defined primer and primer attachment fields.
User-Defined Primers	Enter user-defined primer sequences or a primer from the oligo database. The search engine checks the compatibility of the primers according to primer parameters.


Primer Tab Parameters – Find Primers Dialog Box	
Attach to 5' Terminus of (Anti)Sense Primer	Enter a short (≤ 18 bp) nucleotide sequence (if any) to be attached to the 5' end of either primer, or choose from recognition sites of database RENS. (To select RENS to add, click the Browse button ().) This sequence, while considered in primer parameters, does not affect the calculation of complementarity between primer and molecule. A sequence can be attached to the primer whether or not the primers are user-defined or designed by Vector NTI.

Table 21. 2 Primer tab parameters – Find Primers dialog box

Note: The calculation for T_m is dependent on primer and salt concentrations; varying these concentrations can greatly affect the T_m for any given primer. Make sure to adjust these parameters according to your reaction conditions when performing your PCR analysis to ensure that you obtain accurate T_m values.

Amplicon Tab

Press the **Amplicon** tab (Fig. 21.2). The options on this tab allow you to customize parameters relating to the resulting PCR product. %GC content for the product or a portion of the product and allowed bases adjacent to the primer annealing site can be specified.

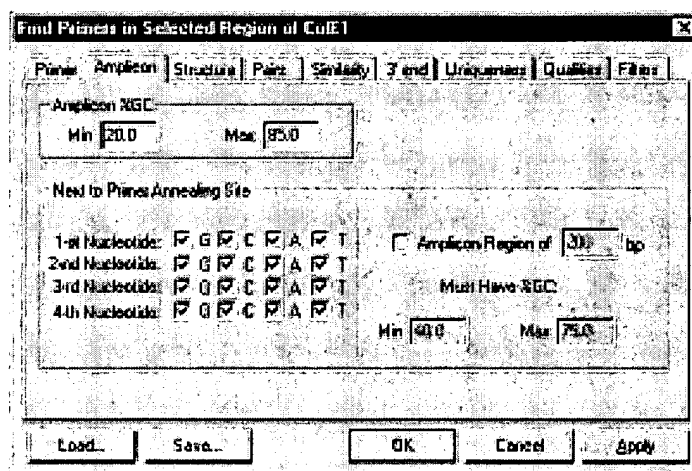


Fig. 21. 2 Amplicon tab on Find Primers dialog box

Amplicon Tab Parameters – Find Primers Dialog Box	
Amplicon %GC	Enter the minimum and maximum for the desired %GC content in the PCR product.
Next to Primer	Choose accepted bases for the four successive bases

Amplicon Tab Parameters – Find Primers Dialog Box	
Annealing Site	adjacent to the primer annealing site. Set minimum and maximum %GC range for a specified length of the amplicon adjacent to the primer annealing site.

Table 21. 3 Amplicon tab parameters – Find Primers dialog box

Structure Tab

Press the **Structure** tab (Fig. 21.3). The options on this tab set acceptable limits for nucleotide repeats, palindromes and hairpin loops for the primers. You can also check your primers/product for a selected group of restriction sites from this tab.

Fig. 21. 3 Structure tab on Find Primers dialog box

Structure Tab Parameters – Find Primers Dialog Box	
Nucleotide Repeats	Enter the maximum permitted length of nucleotide repeats in primers.
Palindromes	Enter the maximum permitted length of palindromes in primers.

Structure Tab Parameters – Find Primers Dialog Box	
Hairpin Loops	Stem Length: Enter the minimum number of base pairs in a hairpin stem. (This value is also used as a minimum stacking length for primer-primer complementarity and primer-primer 3' end complementarity.) Permitted with dG: Check the Permitted box for hairpin loops; enter the minimum permitted value for free energy of hairpin loops. Primers with hairpin loops which have free energy values \geq to this number will be accepted.
Check Hairpin Loops, Palindromes, Nucleotide Repeats and Dimers Only Within 3' Region of ...	Check this box and enter the length of a 3' region if all of a primer's features (repeats, palindromes, hairpin loops, dimers) should be checked only within that 3' region. (If this box is empty, the whole primer will be evaluated.)
Check Primers For Restriction Sites From	Check to find possible cloning sites inside primers and attached nucleotide sequences (if any). In the drop-down menu, specify the REN subbase. Enzymes will be checked for the presence of their sites in the primers and attached sequences, and within the PCR product.

Table 21. 4 Structure tab parameters – Find Primers dialog box

Pairs Tab

Press the **Pairs** tab (Fig. 21.4). Options on this tab specify how closely parameters such as T_m and %GC, etc. must match between two primers in a generated primer set.

Fig. 21. 4 Pairs tab on Find Primers dialog box

Pairs Tab Parameters – Find Primers Dialog Box	
T _m Difference	Enter difference in degrees Celsius between T _m for sense and antisense primers.
%GC	Enter the difference between GC percentages for sense and antisense primers.
Primer-Primer Complementarity	Check the Permitted box for primer-primer complementarity; enter the minimum permitted value for duplex free energy.
Primer-Primer 3' End Complementarity	Check the Permitted box for primer-primer 3' end complementarity; enter the minimum permitted value for duplex free energy.

Table 21. 5 Pairs tab parameters – Find Primers dialog box

Similarity Tab

Press the **Similarity** tab (Fig. 21.5). The options on this tab determine the similarity relationship between the primers and the target sequence.

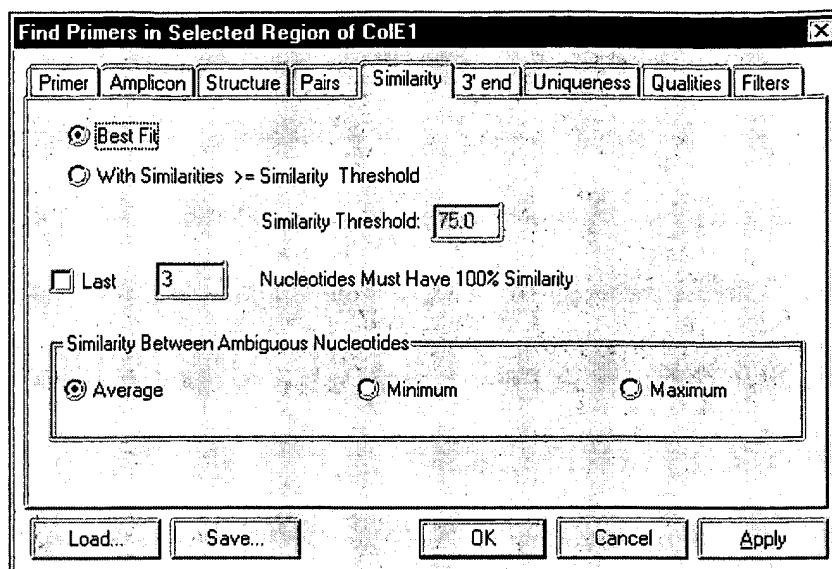


Fig. 21. 5 Similarity tab on Find Primers dialog box

Similarity Tab Parameters – Find Primers Dialog Box	
Best Fit	Check this button to specify the search for site(s) with maximum similarity with no set threshold.
With Similarities > = Similarity Threshold	Check this button to indicate similarity site search above the specified similarity threshold.
Similarity Threshold	Enter the percentage of minimally acceptable similarity.
Last ... Nucleotides Must Have 100% Similarity	Check and specify the number of nucleotides necessary to have 100% complementarity with the target sequence at the 3' end.
Similarity Between Ambiguous Nucleotides	The Average, Minimum, and Maximum buttons specify the acceptable similarity between an ambiguous nucleotide pair. For instance, if you are calculating similarity between N and A, the average similarity is 25%, the minimum similarity is 0%, and the maximum similarity is 100%. In case of R and A they are 50%, 0%, and 100%; in case of R and T - 0%, 0%, and 0%. See similarity chart on page 274.

Table 21. 6 Similarity tab parameters – Find Primers dialog box

3' End Tab

Press the **3' end** tab (Fig. 21.6). The options on this tab allow you to set specifications for the 3' end of the primers generated by VNTI. Parameters such as dG and specific nucleotide content for the 3' end of both sense and antisense primers can be set here.

Fig. 21. 6 3'End tab on Find Primers dialog box

3' End Tab Parameters – Find Primers Dialog Box	
dG <=	Specify the maximum permitted value of 3' end free energy.
Length for Analysis	Enter the length of the primer's 3' region that should be analyzed.
Sense Primer 3' Nucleotides	Check the nucleotide boxes to specify permitted last primer nucleotides for the sense primer.
Antisense Primer 3' Nucleotides	Check the nucleotide boxes to specify permitted last primer nucleotides for the antisense primer.

Table 21. 7 3' End tab parameters – Find Primers dialog box

Uniqueness Tab

Press the **Uniqueness** tab (Fig. 21.7). The options on this tab determine the uniqueness of the primers generated with respect to the PCR product. These parameters can be used to help ensure that generated primers bind to the desired template area with greater specificity than to the rest of the PCR product.

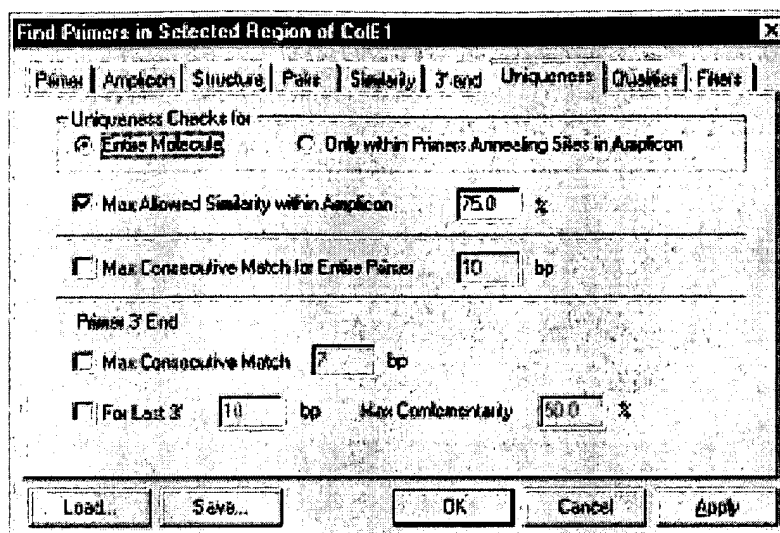


Fig. 21. 7 Uniqueness tab on Find Primers dialog box

Uniqueness Tab Parameters – Find Primers Dialog Box	
Uniqueness Checks for	Choose the area of the molecule to check for primer uniqueness. Either the entire molecule or the Amplicon only can be selected for the uniqueness check.
Max Allowed Similarity with Amplicon	Check this box and enter the similarity threshold to check primer uniqueness on the molecule. Primers which have parasitic hybridization with similarity > /= this threshold will be rejected. Note: this similarity threshold must be </= the minimum similarity required for hybridization of user-defined primers (if any).
Max Consecutive Match for Entire Primer	Check this box and enter the maximum acceptable match of consecutive bases for the entire primer and the Amplicon.
Primer 3' End	Check the first box and enter the number of consecutive 3' bases that must match the amplicon with 100% similarity. Check the second box and specify the maximum acceptable % match between the Amplicon and the designated number of bases on the 3' end of the primer.

Table 21. 8 Uniqueness tab parameters – Find Primers dialog box

Qualities Tab

Press the **Qualities** tab (Fig. 21.8). These parameters govern primer quality by determining how much weight should be assigned parameters specified on the other tabs of the Find Primers dialog box. These values affect scoring functions that evaluate the quality rating of the primer sets generated.

The screenshot shows a dialog box titled "Find Primers in Selected Region of ColE1". It has several tabs: "Primer", "Amplicon", "Structure", "Pairs", "Similarity", "3' end", "Uniqueness", "Qualities", and "Filters". The "Qualities" tab is selected. Inside the dialog, there is a text box stating: "Importance" below must be between 1 and 10: 1 - not important (almost don't care) 10 - crucially important (disregard if not so). Below this, there are two sections. The first section is "Importance to be inside specified limits:" and contains four input boxes: "Tm:" (value 9), "Tm Difference:" (value 9), "%GC:" (value 9), and "%GC Difference:" (value 9). The second section is "Importance to satisfy specified conditions:" and contains five input boxes: "Palindromes:" (value 9), "Nucleotide Repeats:" (value 9), "Hairpin Loops:" (value 9), "Dimers:" (value 9), and "3' End:" (value 9). At the bottom of the dialog are five buttons: "Load...", "Save...", "OK", "Cancel", and "Apply".

Fig. 21. 8 Qualities tab on Find Primers dialog box

The importance factors are integers between 1 to 10 used in calculating the score evaluating primer/oligo quality. The lower the factor, the less weight given in the calculation. For example, for minimal importance, enter 1 in the appropriate box. For maximum importance, enter 10.

Filters Tab

Press the **Filters** tab (Fig. 21.9). On this tab, you can select features that should either be excluded or included as regions to be considered for primer design. For example, if your sequence contains Repeat features, you may want to exclude them to obtain primers with greater specificity. Features can be added or removed by pressing the + and - buttons.

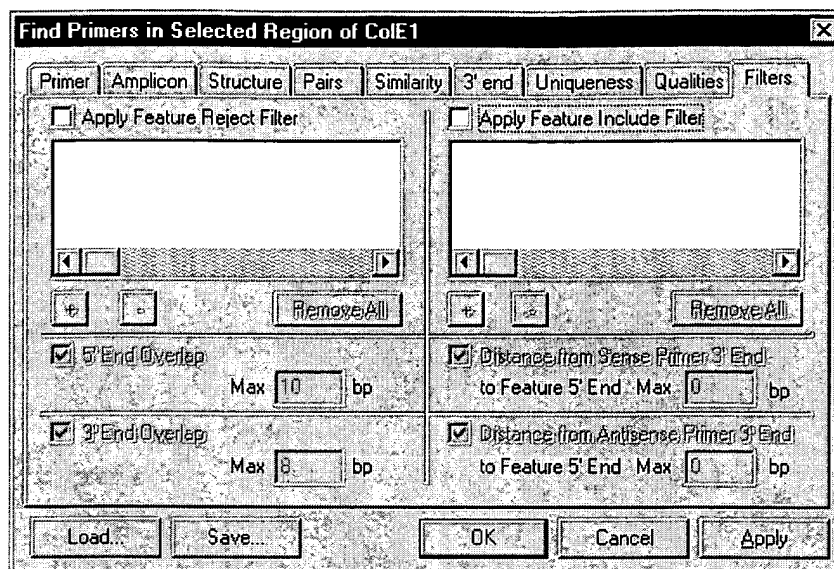


Fig. 21. 9 Filters tab on Find Primers dialog box

Filters Tab Parameters – Find Primers Dialog Box	
Apply Feature Reject Filter	Check the Apply Feature Reject Filter box and press the + and – buttons to add or remove features from the list. This tells VNTI to avoid making primers to the region of the specified feature(s). A maximum allowed overlap of the primer with the feature's 5' and 3' ends can be specified.
Apply Feature Include Filter	Check the Apply Feature Reject Include box and press the + and – buttons to add or remove features from the list. This tells VNTI to include these features in the amplicon when making primers. The maximum acceptable distance from the primer ends to the feature ends can be specified.

Table 21. 9 Filters tab parameters – Find Primers dialog box

Amplify Selection Dialog Box

The Amplify Selection feature is similar to Find PCR Primers except that primer hybridization domains upstream and downstream from the target sequence can be specified. Primers will be generated anywhere within the designated upstream and downstream domains. Like the Find PCR Primers dialog box, pressing the **More>>** button enables complete view. Only the tabs and parameters that differ from the Find PCR Primers dialog box will be discussed here.

Primer Tab

The options in the **Primer** tab (Fig. 21.10) allow you to set parameters for PCR primer search and define primers and/or primer attachments, if desired:

Fig. 21. 10 Primer tab on Amplify Selection dialog box

Primers Tab Parameters – Amplify Selection Dialog Box	
Before	Provides additional upstream region where the Primer may be made.
Amplicon Must Include Region of Molecule	Set the 5' and 3' positions for region of the molecule that must be included in the final amplified product.
After	Provides additional downstream region where the Primer may be made.

Table 21. 10 Primers tab parameters – Amplify Selection dialog box

Amplify Features Dialog Box

Amplify Features is similar to Find PCR Primers except that VNTI will find the best primers for amplifying the designated feature(s) within the region of analysis indicated. Like the Find PCR Primers and Amplify Selection dialog boxes, pressing the **More>>** button enables complete view. Only the tabs and parameters that differ from the Find PCR Primers dialog box will be discussed here.

Primer Tab

The options in the **Primer** tab (Fig. 21.11) allow you to set parameters for PCR primer search and define attachments, if desired. User-defined primers are not allowed:

Fig. 21. 11 Primer tab on Amplify Features dialog box

Primer Tab Parameters – Amplify Features Dialog Box	
Region of Analysis	Specify the 5' and 3' boundaries of the region for primer analysis. Features to be amplified must be within the chosen region. The number of features in the selection is indicated.
Every Amplicon Length	Specify the range for acceptable minimum and maximum product length. VNTI determines primers that will produce products within the set length range.
User-Defined Primers	This feature is NOT present.

Table 21. 11 Primer tab parameters – Amplify Features dialog box

Features Tab

The **Features** tab (Fig. 21.12) allows you to specify the features you want amplified, how many of the chosen features you want in one amplicon and whether primer-feature overlap is permitted:

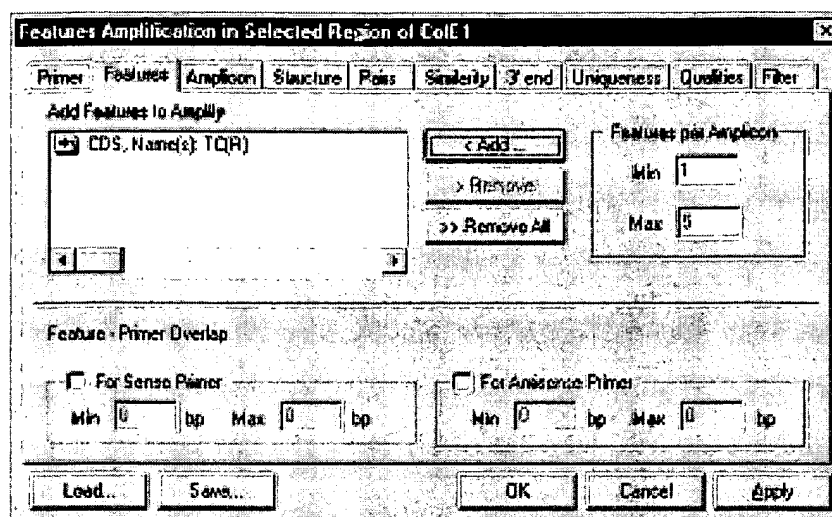


Fig. 21. 12 Features tab on Amplify Features dialog box

Features Tab Parameters – Amplify Features Dialog Box	
Add Features to Amplify	Press the <Add... button to display the feature list. From the list, choose the features you would like to amplify.
Features per Amplicon	Designate the minimum and maximum number of features you want to occur in the amplicons.
Feature-Primer Overlap	To allow feature-primer overlap for the selected features, check the appropriate box/boxes and specify the minimum and maximum permitted base overlap.

Table 21. 12 Features tab parameters – Amplify Features dialog box

Filter Tab

The **Filter** tab (Fig. 21.13) allows you to specify the features you do not want amplified as part of the product:

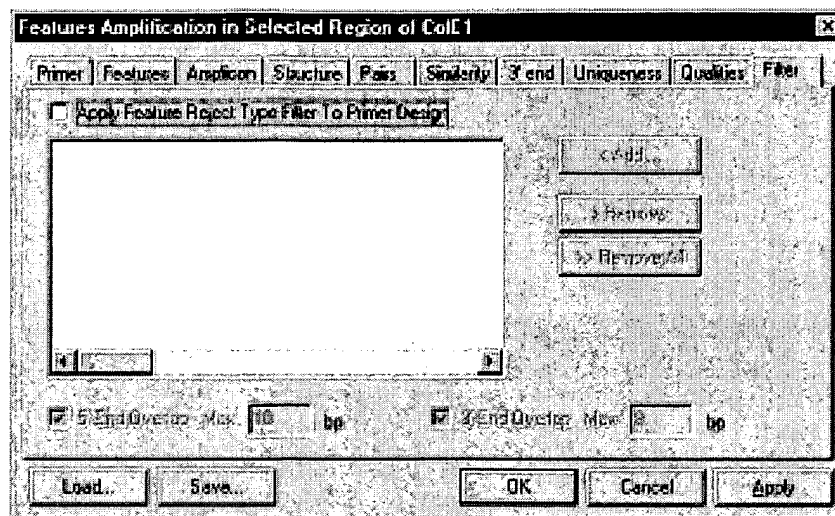


Fig. 21. 13 Filter tab on Amplify Features dialog box

Filter Tab Parameters – Amplify Features Dialog Box	
Apply Feature Reject Type Filter To Primer Design	Check this box to enable the features reject filter. Add or remove features with the <Add..., >Remove... and >Remove All buttons
5' End Overlap	Check the box and specify the maximum permitted overlap of the primer and the rejected feature(s) for the 5' end.
3' End Overlap	Check the box and specify the maximum permitted overlap of the primer and the rejected feature(s) for the 3' end.

Table 21. 13 Filter tab parameters – Amplify Features dialog box

The PCR Analysis Folder

After all of the parameters are defined in the Find PCR Primers, Amplify Selection or Amplify Features box, click **OK** to analyze/design the primers.

During PCR primer analysis, Vector NTI generates a number of primer options that satisfy the conditions defined in the PCR Analysis dialog. These options are inserted into your Molecule Display window's Text Pane as subfolders of the PCR Analysis folder (Fig. 21.14):

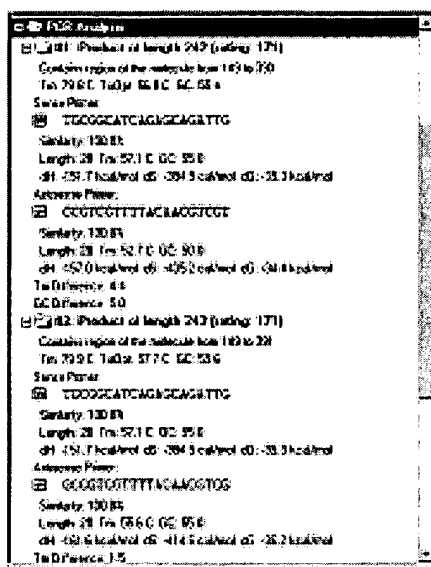



Fig. 21. 14 PCR Analysis folder

In the PCR Analysis Folder, the primer results are sorted in descending order according to their rating values calculated based on the importance factors assigned in the **Qualities** tab. The subfolder titles are numbered and show the length and rating of the PCR products they contain. *The typical maximum rating is 171, although higher ratings are occasionally seen.*

Each subfolder contains:

- The coordinates of molecule's region included in the PCR product.
- Melting temperature for the product
- Optimum annealing temperature for the PCR reaction
- GC percentage of the product.

The next two folder sections describe the sense and antisense primers, each identified at the left with the primer icon ().

- The complementarity between the primer and its target sequence
- The actual primer sequence in the 5'→ 3' direction (with attached sequence, if any)
- The primer length in nucleotides
- The primer melting temperature
- The primer's GC percentage


- Primer values for enthalpy, entropy, and free energy.

If the **Cloning Sites** box was checked in the PCR Analysis dialog box (Fig. 21.1), the primer section also contains a line showing cloning sites found in the product and its primers and attached sequences. A number in parentheses after the cloning site name indicates the number of times that site occurs in the PCR product.


The last line in each product folder shows the difference in the melting temperatures and GC percentages of the sense and antisense primers.

Operations with PCR products

Place the cursor on a PCR product folder line in the Text Pane and open the shortcut menu with its options:

- **Save as Molecule in Database** saves the product as an independent molecule in the database. This option first opens the New DNA/RNA Molecule tabbed dialog box (described in Chapter 20). Enter a name in the text box on the General tab. *You can't tab to the other pages without a name.* The **Description** field on the DNA/RNA Molecule tab automatically defines the origin of the fragment. After entering data, press the **OK** button to save the product to the database.
- **Save to Database and Create Display Window** option saves the fragment in the database and immediately opens it in a display window. The option first opens the New Database Molecule dialog box, described immediately above. After entering information, press **OK**. The molecule is saved to the database and is immediately loaded into a Molecule Display window for your review.
- **Find PCR Product** option selects in the Graphics Pane and Sequence Panes the exact region of the molecule included in the PCR product. You can perform the same operation by pressing the **Find** button () with the product folder selected or select **Edit > Find PCR product**.

Operations with PCR Primers

Wherever you see the oligo symbol to the left of a primer sequence, () , you can perform various oligo functions. Place the mouse cursor on the oligo line and open a shortcut menu with these options:

- **Analyze** opens the Oligo Analysis dialog box (Fig. 21.12) for performing oligo analysis.
- **Add to Oligo List** adds the primer to the Oligo List (Fig. 21.10). This may be useful if you want to investigate possible duplexes between sense and antisense primers, for example.
- **Save To Database** stores it in the database for future use.

Sequencing Primers

To find primers for sequencing a DNA molecule fragment when the molecule is opened in a DNA Molecule Display window, select the target sequence of the molecule—the region to be sequenced. If the sequencing region is long enough, it is divided by Vector NTI into sequencing domains, areas in which a single sequencing reaction will take place. The size of the primer hybridizing domain may then be set (the region in which primers are sought) as well as other desired primer parameters. Several primer options are evaluated and sorted from best to worst.

To prepare for sequencing primer design, open a sequence or Molecule Display window for the molecule and select the region for analysis, using ordinary selection techniques (described in Chapter 3).

Select **Analyze > Sequencing Primers**. *This command is disabled if no selection zone is defined.* This opens the Sequencing Primers dialog box (Fig. 21.15).

Sequencing Primers Dialog Box

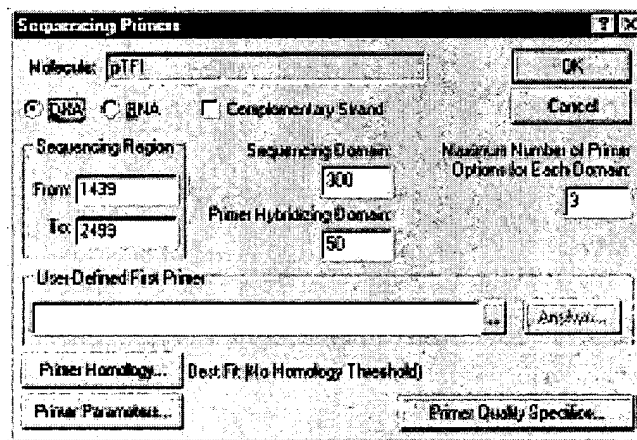


Fig. 21. 15 Sequencing Primers dialog box

The options in this dialog box allow you to set parameters for sequencing primer search:

Sequencing Primers Dialog Box Parameters	
DNA/RNA	Select the type of nucleotide sequence.
Sequencing Region	Enter the start and end coordinates of the sequencing region.
Sequencing Domain	Enter the number of bases for a single sequencing reaction.

<i>S</i> equencing Primers Dialog Box Parameters	
Primer Hybridizing Domain	Enter the length of region where primers for each sequencing domain should be sought.
Maximum Number of Primer Options for Each Domain	Enter the number of primers to be found for each sequencing domain. (The actual result may contain fewer primers than this number if there are not enough possible primers.)
User-Defined First Primer	Enter a user-defined nucleotide sequence to be evaluated as a primer for the FIRST sequencing domain instead of leaving primer search to Vector NTI.
Analyze	Press the Analyze button to analyze a specified primer in the Oligo Analysis dialog box (Fig. 21.12).
Primer Similarity	Press the Primer Similarity button to specify similarity requirements in the Similarity of sequencing Primers dialog box (Fig. 21.2) . (See table 21.3)
Primer Parameters	Press the Primer Parameters button to set parameters for sequencing primers in the Primer/Oligo Parameters dialog box (Fig. 21.3). (See Table 21.4 for description.)
Primer Quality Specifics	Press the Primer Quality Specifics button to open the Primer/Oligo Quality Specifics dialog box (Fig. 21.4) for assigning "importance factors" to sequencing primers.

Table 21. 14 Sequencing Primers dialog box parameters

After all parameters are defined, click **OK** to search for the primers. During primer analysis, Vector NTI generates a number of primer options that satisfy the conditions you have defined. The primer options appear in the Text Pane as subfolders (Fig. 21.14).

Primer Similarity Dialog Box

Press the **Primer Similarity** button in the [Sequencing Primer] [Hybridization Probe] Analysis box to open this dialog box to specify similarity requirements for the primer(s)/probe (Fig. 21.16).

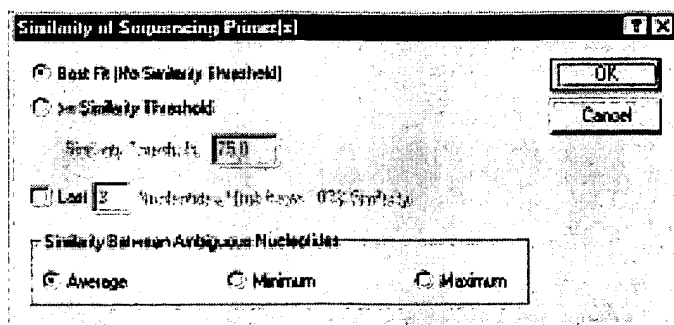


Fig. 21. 16 Primer Similarity dialog box

Primer Similarity Dialog Box Parameters	
Best Fit	Check this button to specify the search for site(s) with maximum similarity with no set threshold.
> = Similarity Threshold	Check this button to indicate similarity site search above the specified similarity threshold.
Similarity Threshold	Enter the percentage of minimally acceptable similarity.
Last ... Nucleotides Must Have 100% Similarity	Check and specify the number of nucleotides necessary to have 100% complementarity with the target sequence at the 3' end.
Similarity Between Ambiguous Nucleotides	The Average, Minimum, and Maximum buttons specify the acceptable similarity between an ambiguous nucleotide pair. For instance, if you are calculating similarity between N and A, the average similarity is 25%, the minimum similarity is 0%, and the maximum similarity is 100%. In case of R and A they are 50%, 0%, and 100%; in case of R and T - 0%, 0%, and 0%. See similarity chart on page 274.

Table 21. 15 Primer Similarity dialog box parameters

Primer/Oligo Parameters Dialog Box

Press the **Primer Parameters** button in the [Sequencing Primer] [Hybridization Probe] Analysis box to open this dialog box to specify acceptable limits for the interaction of the primer(s) or hybridization probes (oligo) with the selected target sequence (Fig. 21.17).

Primer/Oligo Parameters

dG Temperature (C) Hairpin Loop Stem Length

Probe Conc. (pMol) Palindromes <=

Salt Conc. (mMol) Nucleotide Repeats <=

% Formamide

Length: to Tm: to C

%GC: to C

3' End: to C

dG <= kcal/mol

Length for Analysis:

3' Nucleotide: ☒ G ☒ C ☒ A ☒ T

Hairpin Loops: ☒ Permitted with dG >=

Primer-Primer Complementarity: ☒ Permitted with dG >=

Primer-Primer 3' End Compl.: ☒ Permitted with dG >=

☒ Check All Features Only Within 3' Region of bp

☒ Primer/Oligo Must be Unique With Similarity %

Fig. 21. 17 Primer/Oligo Parameters dialog box

Primer/Oligo Parameters	
dG Temperature	Enter the temperature in degrees Celsius to be used for calculating free energy values.
Probe Concentration	Enter the value of probe concentration in pMol, if known.
Salt Concentration	Enter the value of salt concentration in mMol, if known.
% Formamide	When hybridizing in formamide, enter the formamide concentration in %.
Length	Enter primer length limits. Note: Nucleotide sequences such as RENs attached to a primer's 5' end are included when calculating primer length.
%GC	Enter the limits of G/C percentage in the primer and the difference between GC percentages for sense and antisense primers.
Tm	Enter limits in degrees Celsius for primer melting temperature (T_m) (temperature at which 50% of primer is a duplex) and the difference between T_m for sense and antisense primers.

Primer/Oligo Parameters	
3' End	Specify parameters for the primer's 3' end. In the Length for Analysis field, enter the length of the primer 3' region that should be analyzed. In the dG <= field, specify the maximum permitted value of 3' end free energy. Check the nucleotide boxes to specify permitted last primer nucleotides.
Hairpin Loop Stem Length > =	Enter the minimum number of base pairs in a hairpin stem. (This value is also used as a minimum stacking length for primer-primer complementarity and primer-primer 3' end complementarity.)
Palindromes <=	Enter the maximum permitted length of palindromes in primers.
Nucleotide Repeats <=	Enter the maximum permitted length of nucleotide repeats in primers.
Hairpin Loops	Check the Permitted box for hairpin loops; enter the minimum permitted value for free energy of hairpin loops. Primers with hairpin loops which have free energy values > /= to this number will be accepted.
Primer-Primer Complementarity	Check the Permitted box for primer-primer complementarity; enter the minimum permitted value for duplex free energy.
Primer-Primer 3' End Complementarity	Check the Permitted box for primer-primer 3' end complementarity; enter the minimum permitted value for duplex free energy.
Check All Features Only Within 3' Region of ...	Check this box and enter the length of a 3' region if all of a primer's features (repeats, palindromes, hairpin loops, dimers) should be checked only within that 3' region. (If this box is empty, the whole primer will be evaluated.)
Primers/Oligos Must Be Unique With Similarity ...	Check this box and enter the similarity threshold to check primer uniqueness on the molecule. Primers which have parasitic hybridization with similarity > /= this threshold will be rejected. Note: this similarity threshold must be </= the minimum similarity required for hybridization of user-defined primers (if any).

Table 21. 16 Primer/Oligo parameters

Primer/Oligo Quality Specifics Dialog Box

Press the [Primer]/[Oligo] Quality Specifics button in the [Sequencing Primer] [Hybridization Probe] Analysis box to open this dialog box to assign "importance factors" to different primer/oligo parameters (Fig. 21.18).

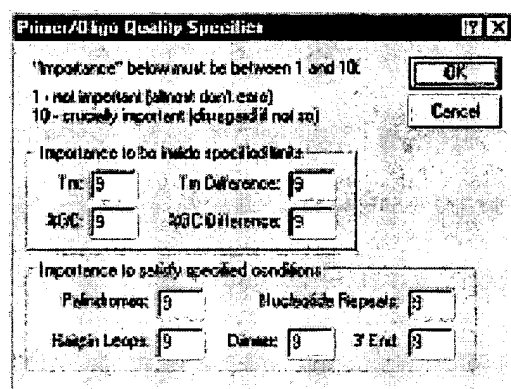


Fig. 21. 18 Primer/Oligo Quality Specifics dialog box

The importance factors are integers between 1 to 10 used in calculating the score evaluating primer/oligo quality. The lower the factor, the less weight given in the calculation. For example, for minimal importance, enter 1 in the appropriate box. For maximum importance, enter 10.

The Sequencing Primers Folder

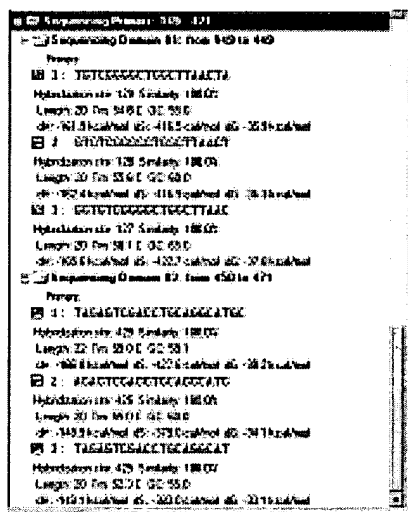



Fig. 21. 19 Sequencing Primers folder

The Sequencing Primers folder (Fig. 21.19) contains one subfolder with primers for each sequencing domain in the analyzed region. Subfolder titles are numbered and show the length and boundaries of the sequencing domains. Each subfolder contains:

- The primer sequence, in the 5' → 3' direction
- The primer hybridization site and primer similarity for that site
- The primer length in nucleotides
- Melting temperature for the primer
- GC percentage of the primer
- Values for enthalpy, entropy, and free energy

Wherever you see the oligo symbol to the left of a primer sequence, () , you can perform various oligo functions. The shortcut menu associated with these primers, launched by right clicking on the primer sequence, has these options:

- **Analyze** opens the Oligo Analysis dialog box (Fig. 21.12) to perform oligo analysis.
- **Add to Oligo List** adds the primer to the Oligo List (Fig 21.10). This may be useful if you want to investigate possible duplexes between sense and antisense primers, for example.
- **Save To Database** stores it in the database for future use.

Hybridization Probes

Hybridization probes analysis is used to design oligonucleotides that will hybridize with a selected molecule fragment, within specified parameters. Vector NTI can generate a set of oligos or use user-defined or database-stored oligos to test for hybridization efficiency with a target molecule.

Open a Molecule Display window for the molecule and select the region for analysis, using ordinary selection techniques (described in Chapter 3).

Select **Analyze > Hybridization Probes**. *This command is disabled if no selection zone is defined.* This opens the Hybridization Probes dialog box (Fig. 21.20).

Hybridization Probes Dialog Box

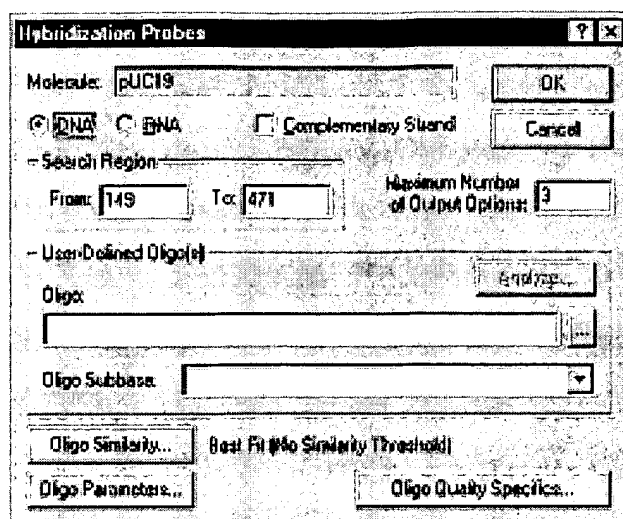


Fig. 21. 20 Hybridization Probes dialog box

Set parameters in this dialog box for successful hybridization of your probes with your target fragment:

Hybridization Probes Dialog Box Parameters	
Search Region	Enter the start and end coordinates of the region where oligos should be hybridized:
DNA/RNA	Select the type of nucleotide sequence.
Maximum Number of Output Options	Enter the number of oligos you would like to find. The actual result may contain fewer oligos than this number if there are not enough possible oligos.
User-Defined Oligo(s)	Enter an oligo's nucleotide sequence or choose an oligo from the oligo database to check hybridization qualities of a specific oligo. Enter oligo subbase name or choose a name from oligo subbases if you want to check hybridization qualities of all oligos in this subbase.
Analyze	Press the Analyze button to analyze a specified oligo. This button brings up the Oligo Analysis dialog box (Fig. 21.12).
Oligo Similarity	Press the Oligo Similarity button to open the Probe Similarity dialog box (Fig. 21.2) to specify similarity requirements. (See Table 21.3)

Hybridization Probes Dialog Box Parameters	
Oligo Parameters	The Oligo Parameters button opens the Primer/Oligo Parameters dialog box (Fig. 21.3). for setting parameters for probes. (See Table 21.4 for description.)
Oligo Quality Specifics	Press the Oligo Quality Specifics button to open the Primer/Oligo Quality Specifics dialog box (Fig. 21.4) for assigning "importance factors" to probes.

Table 21. 17 Hybridization Probes dialog box parameters

The Hybridization Probes Folder

Hybridization probe analysis generates oligos that will hybridize with the target sequence and stores them in the Hybridization Probes folder (Fig. 21.21) in the Text Pane.

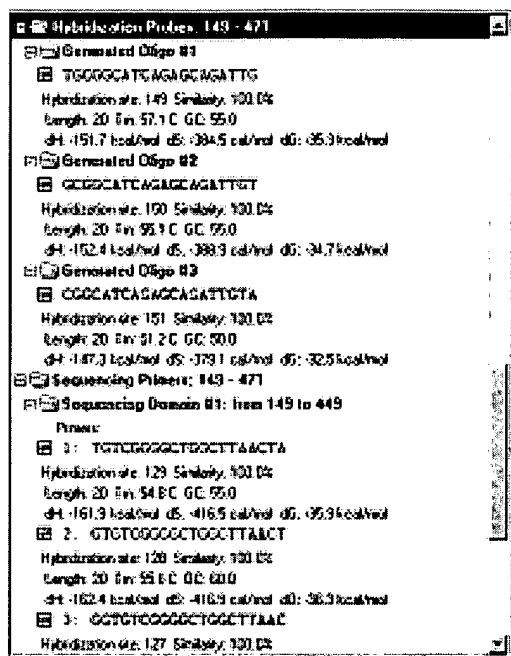



Fig. 21. 21 The Hybridization Probes folder

If a user-defined oligo was specified, the Hybridization Folder contains only that oligo, if it could hybridize in the defined conditions. The Hybridization folder contains one subfolder for each probe for the analyzed region.

Subfolder titles show the name of the oligo contained within.

For each oligo, the subfolders contain:

- The nucleotide sequence of the probe in the 5'-3' direction
- The oligo hybridization site and similarity
- The oligo length in nucleotides
- The oligo melting temperature
- The oligo GC percentage
- Oligo values for enthalpy, entropy, and free energy.

Wherever you see the oligo symbol to the left of a probe sequence, () , you can perform various oligo functions. Place the mouse cursor on the oligo line and right click to open a shortcut menu with these options:

- **Analyze** opens the Oligo Analysis dialog box (Fig. 21.12) to perform oligo analysis.
- **Add to Oligo List** adds the primer to the Oligo List (Fig 21.10). This may be useful if you want to investigate possible duplexes between sense and antisense primers, for example.
- **Save To Database** stores it in the database for future use.

Oligonucleotide Analysis

This section describes Vector NTI's oligonucleotide functions, including the Oligo List and the Oligo Editor, Oligo Analysis, and Oligo Duplexes dialog boxes.

The Oligo List

The Oligo List is a data clipboard for listing and analyzing oligonucleotides from a display window or from the database. Oligonucleotides can be user-defined or determined by selecting a target sequence and letting VNTI design an oligo for the sequence. From this central list, you can add new oligos or select existing ones for editing and analysis.

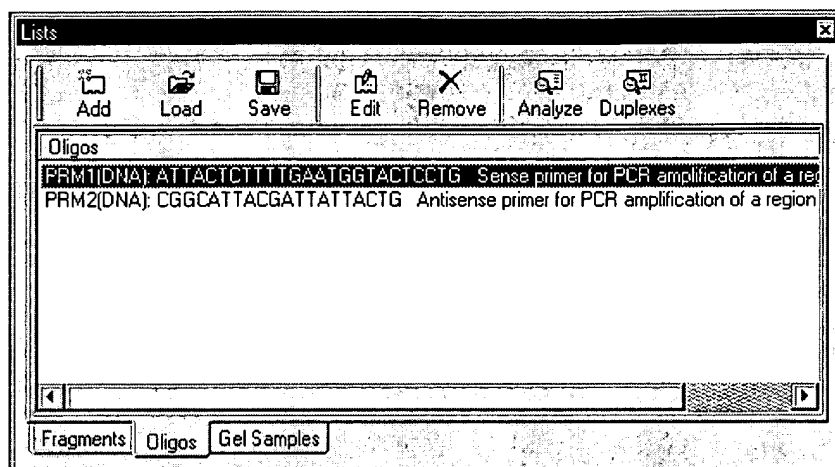



Fig. 21. 22 Oligo List dialog box

To open the Oligo List dialog box (Fig. 21.22), in a Molecule Display window, press the

Show Oligo List button () on the Main Toolbar or choose **List > Oligo List**. Buttons in the box are summarized as follows:


Oligo List Dialog Box Parameters	
Add	Opens the Oligo Editor dialog box (Fig.21.23) to enter a new oligo to the Oligo list.
Load	Loads oligos into the list from the VNTI oligo database.
Save	Press Save to Database button () to save it in the database.
Edit	Opens the Oligo Editor dialog box (Fig. 21.23) to edit the selected oligo.
Remove	Removes the selected oligo from the Oligo List.
Analyze	Initiates oligo analysis on the selected oligo. (See Fig. 21.24)
Duplexes	Investigates the duplexes on one or more oligos. (See Fig. 21.24)

Table 21. 18 Oligo List dialog box parameters

- In the Nucleotide Sequence box, enter or edit the standard I.U.B. base code characters for the molecule sequence. (See Appendix C.)
- Enter the oligo's description.

User Fields: Modify custom data in a form of fields (see Chapter 18 for User Fields details.)

Comments: Enter text comments about the oligo.

Keywords: To add a keyword for the oligo, type a new word or select an item in the list of existing keywords. Press the **Add** button to move the keyword into the oligo's keyword list. To remove an item from the keyword list, select it and press the **Remove** button.

Exit Oligo Editor by clicking **OK** or **Cancel**.

Oligo Analysis Dialog Box

Oligonucleotides for analysis can be selected by selecting a target sequence and letting VNTI design an oligo for the sequence or entering a user-defined oligo.

- Select a target sequence in an open Molecule Display window, and select **Analyze > Oligo Analysis**; the oligo selected by Vector NTI is displayed in the text box of the Oligo Analysis dialog box (Fig. 21.24) that opens.

or

- Open the Oligo Analysis dialog box first with **Analyze > Oligo Analysis**, and then enter a user-defined oligo sequence in the Oligonucleotide text box.
- You can also select an oligo listed in a Text Pane folder and select **Analyze** from the shortcut menu.

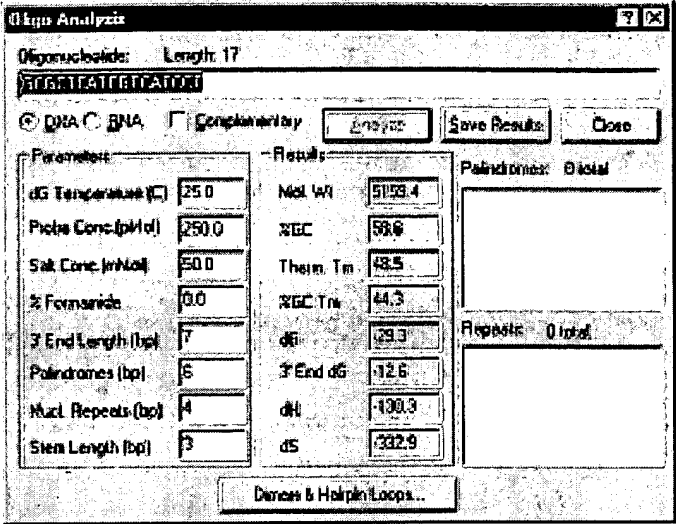


Fig. 21. 24 Oligo Analysis dialog box

Select radio buttons for molecule type, and check **Complementary** to replace the oligo with its complement.

The **Parameters** column lets you accept default settings or specify acceptable limits for the interaction of the oligo with its selected target sequence. Settings are summarized as follows:

Oligo Analysis Parameters	
dG Temperature	Enter the temperature in degrees Celsius to be used for calculating free energy values.
Probe Concentration	Enter the value of probe concentration in pMol.
Salt Concentration	Enter the value of salt concentration in mMol.
%Formamide	When hybridizing in formamide, enter the formamide concentration in %.
3' End Length (bp)	Specify the length of the oligo 3' region which should be analyzed.
Palindromes	Enter the minimum length of palindromes to search for.
Nucleotide Repeats	Enter the minimum length of nucleotide repeats to search for.

Oligo Analysis Parameters	
Stem Length	Enter the minimum number of base pairs in a hairpin or dimer stem.

Table 21. 19 Oligo Analysis parameters

Note: The calculation for T_m is dependent on primer and salt concentrations; varying these concentrations can greatly affect the T_m for any given primer. Make sure to adjust these parameters according to your reaction conditions when performing your oligo analysis to ensure that you obtain accurate T_m values.

After these parameters are set, press **Analyze**. Results are summarized in the same dialog box as follows:

Oligo Analysis Results	
Mol. Wt	Molecular weight of the oligo.
%GC	The percentage of G and C in the oligo.
Therm. T_m	The melting temperature (temperature at which 50% of the oligo is a duplex) calculated by the Nearest-Neighbors method. <i>This is useful for short oligos, < ~35 bp.</i>
% GC T_m	The %GC T _m field shows the melting temperature calculated by the %GC method. <i>This is useful for long oligos, > ~35-40 bp.</i>
dG	The free energy values of the entire oligonucleotide.
3' End dG	The free energy of the 3' end of the oligo.
dH	The enthalpy of the entire oligo.
dS	The entropy of the entire oligo.
Palindromes	Examine the palindromes in the oligo.
Nucleotide Repeats	Examine the nucleotide in the oligo.

Table 21. 20 Oligo Analysis results

Press the **Dimers & Hairpin Loops** button to examine the oligo's dimers and hairpin loops. *Both are undesirable secondary structures that interfere with hybridization or amplification efficiency.*

Dimers & Hairpin Loops Dialog Box

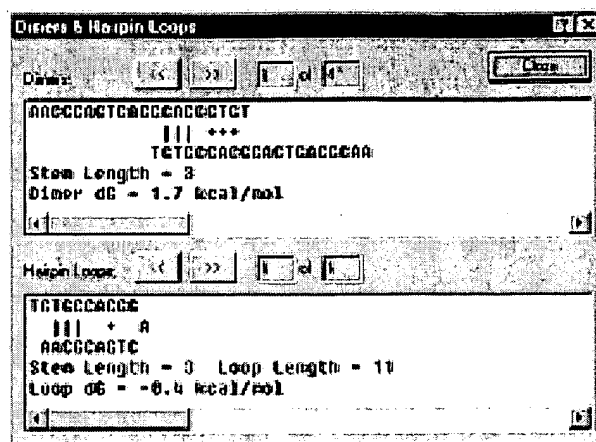


Fig. 21.25 Dimers & Hairpin Loops dialog box

Note: In the graphical depiction of dimers and hairpin loops, vertical lines indicate the primary interaction, based on the stem length set and plus symbols indicate secondary interactions. The greater the dG value, the weaker the interaction; secondary interactions are not considered in the dG calculation.

Dimers

Dimers are formed when one oligo anneals to another oligo. *See the Dimer example in the upper pane of Fig.21.13.* Examine the dimers the oligo contains. Press the **Next** (Next button icon) button to view the next dimer. Press the **Prev** button (Prev button icon) to view the previous dimer.

Hairpin Loops

Hairpin loops are formed when an oligo doubles back on itself. *See the Hairpin Loop example in the lower pane of Fig. 21.25.* Examine the hairpin loops the oligo contains. Press the **Next** button (Next button icon) to view the next hairpin loop. Press the **Prev** button (Prev button icon) to view the previous hairpin loop.

Note: To avoid any of the observed results in your final product, modify the oligo or the parameter settings and re-analyze the oligo.

Oligo Duplexes Dialog Box

This dialog box is opened by pressing the **Duplexes** button in the Oligo List dialog box or by selecting **Analyze > Oligo Duplexes**. The Oligo Duplexes dialog box (Fig. 21.26) allows you to generate all possible duplexes of selected oligonucleotides.

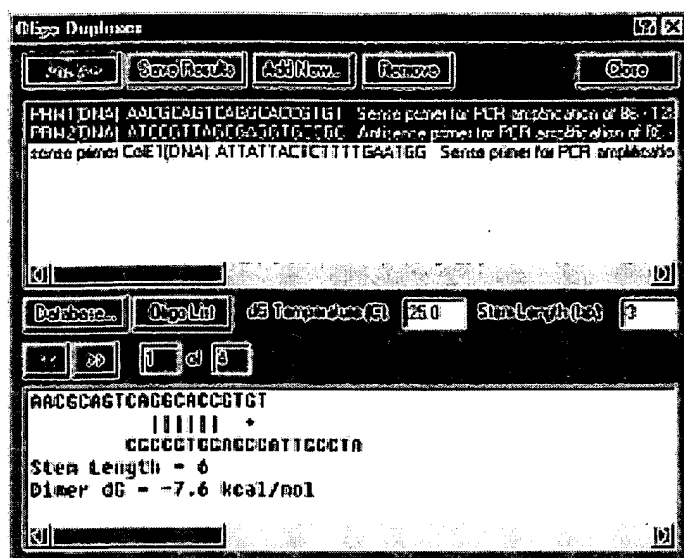




Fig. 21. 26 Oligo Duplexes dialog box

The upper pane shows oligonucleotides that are currently in the workspace. Select one oligo to analyze self-duplexes. Select any two oligos to analyze their cross-duplexes. Options in the dialog box are summarized as follows:

- **Analyze** analyzes duplexes of the selected oligos when one or two oligos are selected, press to generate and. *If less than one or more than two oligos are selected, the Analyze button is disabled.*
- **Save Results:** Save results to the database.
- **Add New** opens the Oligo Editor to enter a new oligo for the Oligo Duplexes workspace.
- **Remove:** removes selected oligos from the workspace.
- **Database:** adds oligos to the workspace from the oligo database.
- **Oligo List:** adds oligos to the workspace from the Oligo List.
- **dG Temperature:** enter the temperature in degrees Celsius to be used for calculating free energy values.
- **Stem Length:** enter the minimum acceptable number of base pairs in a hairpin or dimer stem.

The display pane at the bottom shows the dimers found in oligos selected in the top display window. The stem length and dimer dG are shown. Use the scroll bar to move left or right

along the dimer. Press the **Next** button () to view the next dimer. Press the **Previous** button () to view the previous dimer.

Note: In the graphical depiction of duplexes, vertical lines indicate the primary interaction, based on the stem length set and plus symbols indicate secondary interactions. The greater the dG value, the weaker the interaction; secondary interactions are not considered in the dG calculation.

Mutagenesis

In a DNA/RNA Molecule Display window, you can search for “silent mutations” which, in any selected region, do not affect amino acid translation of the selected DNA but result in presence or disappearance of one or more restriction sites. You can search separately for direct or complimentary sequence strand mutations.

To search for the silent mutations make sure that Display Setup contains Restriction Map options for an at least one restriction enzyme. Select a region of a molecule in the display window and choose **Analysis > Mutagenesis/Direct (Complementary) Strand**.

In the Choose Restriction Enzymes dialog box that opens, select one or more restriction enzyme sites to be analyzed. Select at least one enzyme.

Note: You can only analyze restriction sites for the enzymes listed in the dialog box. To modify the enzyme selection, select **Analyze > Restriction Sites** to open the Restriction Map Setup dialog box where you can modify the list of RENs in use. For a discussion of Restriction Map Display Setup, see Chapter 19.

Click **OK** to initiate the mutagenesis search. Vector NTI analyzes the selected region of the molecule and attempts to generate suitable silent mutations. The reading frame for amino acids is defined by the start of the selected region so that the first three nucleotides of the selected region form the first codon.

Note: The program is able to find both “single” (just one nucleotide altered) and “multiple” (several neighbor nucleotides altered) mutations for any elementary event (appearing and/or disappearing of at least one site) significantly widening the set of possible solutions compared to just “single”-mutation analysis.

The analysis results are listed in the Mutagenesis folder (Fig. 21.27) of the Text Pane of the display window.

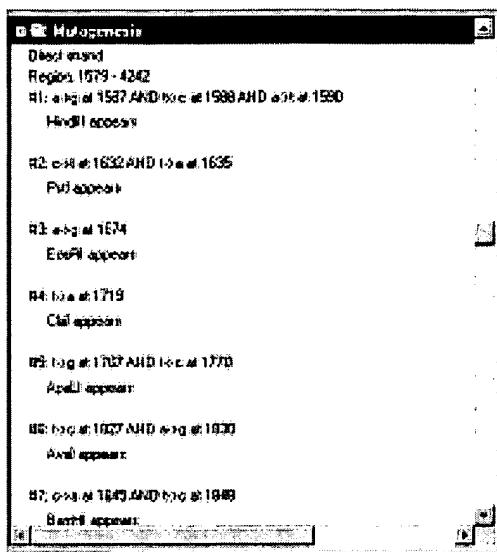



Fig. 21. 27 Mutagenesis folder

The folder contains a list of mutation options that result in the appearing and/or disappearing of at least one restriction site. The options are sorted by the position of the first altered nucleotide. If you selected the complementary strand option, mutation coordinates on both complementary and direct strands are listed.

To identify a mutation region on the molecule for each mutation option, set the cursor on a numbered line under the Region line. Press the **Find** button () , the CTRL + F keys, select **Edit > Find Mutation**, or the corresponding command on the shortcut menu. This locates the nucleotides to be altered according to the mutation option.

Calculating Restriction Fragments

From an open DNA/RNA Molecule Display window, you can calculate restriction fragments that are the result of cutting the molecule with any subbase of enzymes constituting the restriction map.


With a Molecule Display window open, select **Analyze > Restriction Fragments**. In the dialog box that opens, all enzymes are selected by default. Press **Unselect** and select at least one restriction enzyme in the list for “digesting” your molecule.

Note: To modify the enzyme you can use for calculating restriction fragments, select **Analyze > Restriction Sites** to open the Restriction Map Setup dialog box. Here you can change the list of RENs in use. For a discussion of Restriction Map Display Setup, see Chapter 19.

Vector NTI calculates the appropriate restriction fragments and lists the results in the Restriction Fragments folder of the Text Pane.


To easily identify a restriction fragment on the molecule map in the Graphics Pane, set the cursor on the appropriate line in the Restriction Fragments folder. Press the **Find** button



() or select **Edit > Find Fragment** or the corresponding command on the shortcut menu.

ORFs and Motifs Search

You can use Vector NTI tools to search for ORFs and user-defined motifs on DNA molecules. Both searches are launched from setup dialog boxes where you define parameters for the search. To initiate either search, in a Molecule Display window select

Analyze > Orfs or **Analyze > Motifs** or press the **Display Setup** button () and select **Display Setup** from the dropdown menu. In the Display Setup dialog box, press the **Motifs Setup** or **ORFs Setup** button, opening the corresponding dialog box. The parameters in these boxes are discussed in depth in Chapter 19.

After setting parameters, click **OK** to launch either search.

ORFs Search Results

ORFs search results are displayed in a Text Pane folder, listing the ORF regions meeting the criteria specified in the search. ORFs are displayed as black arrows in the Graphics Pane. Phase indicators in the left margin of the Sequence Pane show the phases of the ORFs. For the direct strand, the phase is determined by the position of the first select nucleotide relative to the beginning of the molecule. For the complementary strand, the phase is determined by the position of the last selected nucleotide relative to the end of the molecule. An example of ORFs search results is shown in Fig. 21.28. To eliminate the ORF arrow display, return to the Display Setup dialog box and uncheck ORFs Setup.

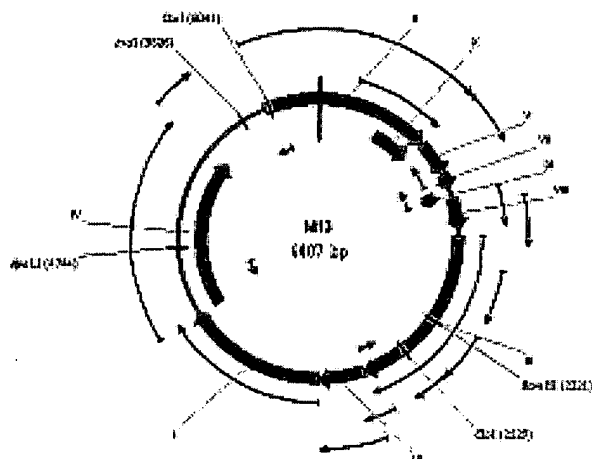


Fig. 21. 28 ORFs search results

Motifs Search Results

Motifs found in a motifs search are displayed in a Motifs folder in the Text Pane and concurrently on the molecule map in the Graphics Pane (not shown). The shortcut menu that opens from a selected motif lists three commands specific to motifs:

- **Motif Site Properties** displays the site and the percent similarity of the query motif with that found on the molecule at that site
- **Add Motif Site to F(eature)Map** opens the Molecule Feature dialog box (Fig. 20.7) where you can name and categorize the motif before adding it to the molecule.
- **Find Motif site** (in the Graphics Pane) positions the caret at the motif site on the molecule map.

Back Translation

The Back Translation feature of the Vector NTI Suite allows you to obtain a DNA sequence from a protein sequence by reversing the translation process. The codon usage can be set by selecting any of the options from the drop down menu in the Back Translation dialog box. The ambiguity is defined in a codon usage table. There are a variety of pre-set codon usage settings available.

Access to the Back Translation dialog box is through the Analyze menu option of most Vector NTI Suite applications where proteins can be displayed or selected. You can also open Back Translation through the Vector NTI Suite program files. From the Back Translation dialog box, select **File > Open** to open previously saved protein documents into the Back Translation dialog box (Fig. 21.29).

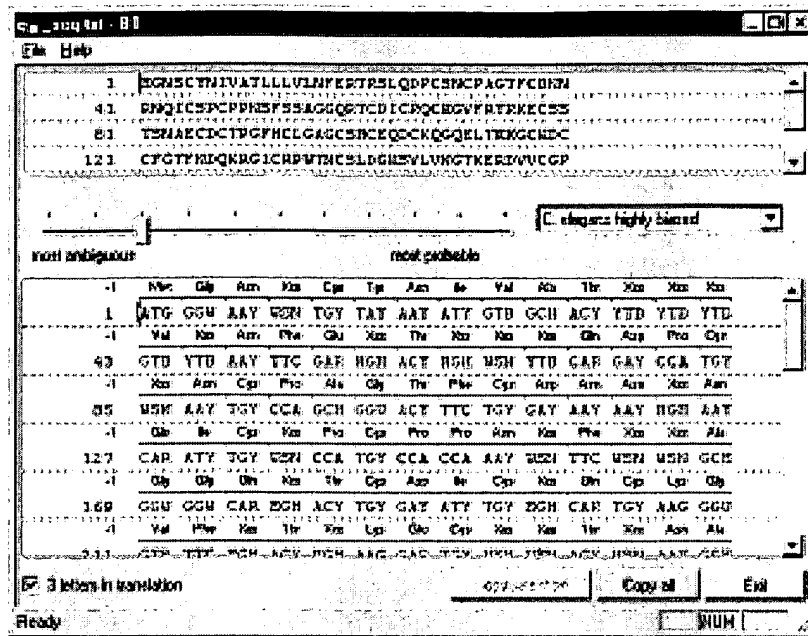


Fig. 21. 29 Back Translation dialog box

To vary the level of codon ambiguity, slide the slider from "Most ambiguous" to "Most Probable" or any setting in between. The resulting sequence can be copied by pressing the **Copy All** or **Copy Selection** buttons at the bottom of the screen. The resulting sequence can be pasted in a new molecule in Vector NTI.

Broadcast Selection

The Broadcast Selection feature of the Vector NTI Suite allows you to select a region in the sequence pane of an opened molecule in one Vector Suite application and then transfer that selection to the same molecule that is currently open in other Vector NTI Suite applications.

The Broadcast Selection feature can be accessed from the shortcut menu visualized by right clicking on a selection in the sequence pane of any of the Vector NTI Suite applications (Fig. 21.30):

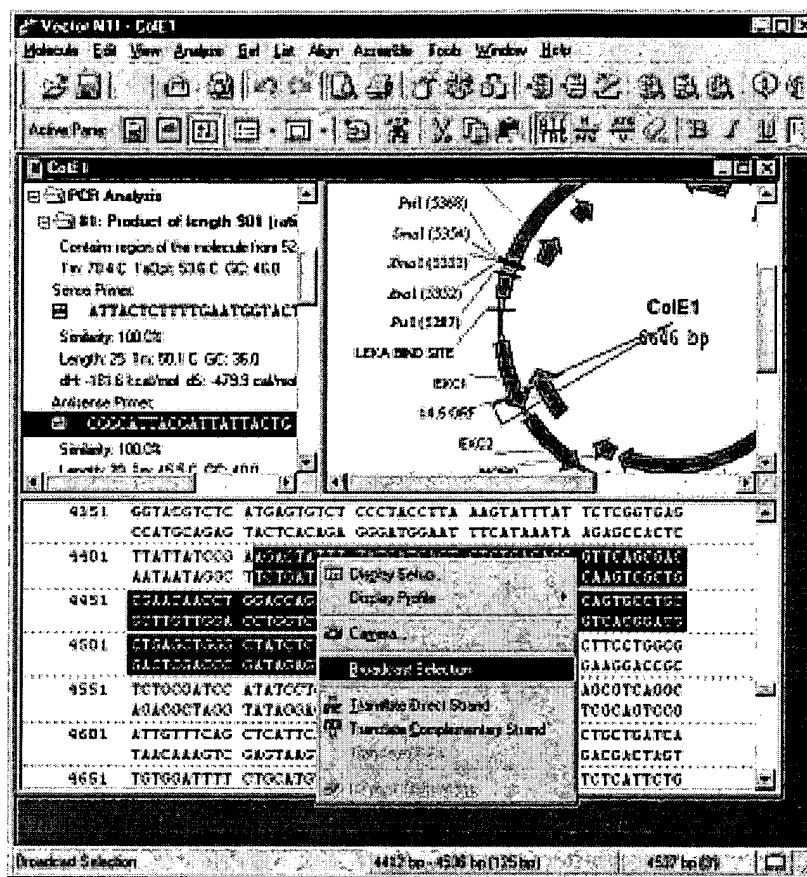


Fig. 21. 30 Broadcast Selection feature

Choose the Broadcast Selection entry from the shortcut menu to mark that sequence area in the same molecule currently open in any other Vector NTI Suite application.

Chapter 22 Molecule Construction

Introduction

This chapter describes the essentials of creating new molecules with Vector NTI. Features of two important dialog boxes used for molecule creation—the Construct/Design Molecule dialog box, and the Molecule Editor—are discussed

This chapter covers the following points:

- descriptions of molecule types: basic and constructed
- differentiating in Vector NTI between molecule Construction and Design
- preparing fragments for Construction using Fragment Wizard
- using the Construct/Design Molecule dialog box as a tool for molecule creation
- modifying component fragments termini with biochemical operations
- using Molecule Editor to prepare molecule fragments
- importing molecules

Molecule Types: Basic And Constructed

Molecules in Vector NTI can be of two fundamental types: *basic* and *constructed*.

Basic molecules are not built from component fragments. Their sequences, Feature map, and restriction maps of unsequenced regions (DNA molecules only) are entered by the user or imported from other databases.

Constructed molecules are built from one or more fragments. For DNA molecules, these can be fragments of existing DNA molecules, linkers, adaptors, etc. The Feature map and nucleotide sequences of parent molecules are automatically transferred to a constructed DNA molecule. You can add features to the Feature map in addition to those obtained from the ancestors. Constructed DNA molecules are created by Construction or Design, Vector NTI terms for recombinant processes that are differentiated below.

Protein molecules can be constructed by translating a certain region of a DNA molecule. A constructed protein molecule does not receive any Feature map from its parent DNA molecule.

Methods of creating new DNA/RNA molecules

In Vector NTI, there are four different ways of creating new DNA/RNA molecules:

Basic Molecules:

Importing molecules or sequences (including their feature tables) in the following formats: GenBank, GenPept, EMBL, SWISS-PROT, FASTA, and ASCII text. You can also import molecule data from “quasi-GenBank” files (that is, GCG’s “GenBank” output files). Molecule import is discussed on page 235.

Creating new molecules “from scratch.” You can define a molecule’s nucleotide or amino acid sequence by hand or paste it from the clipboard and enter the sequence as a new molecule, describing the feature and restriction maps if you wish. This is described in Chapter 20.

Constructed Molecules:

Construction of new DNA/RNA molecules from compatible components fragments from other molecules, linkers, adaptors, etc. In Construction the fragments and restriction sites are entirely defined the user.

Design of new DNA/RNA molecules from components also in a user-defined fragment list. In Design, however, instead of personally choosing the restriction sites, methods of terminus modification, etc., the user turns the Design process over to Vector NTI. The software takes advantage of the best possible restriction sites and genetic engineering techniques to design the recombination process.

Tools for Creating New DNA/RNA Molecules

For creating new molecules, Vector NTI must be in Molecule Editing mode, the default mode when the Molecule Display window opens.

Vector NTI has three important tools for creating new constructed molecules: the Fragment Wizard, the Construct/Design Molecule dialog box and the Molecule Editor. The process of creating a molecule generally follows these steps, in an active Graphics Pane:

1. Define the component fragments for the recombinant molecule using Fragment Wizard.
2. Add defined fragments to the Goal Molecule Definition List.
3. Open the Construct/Design Molecule dialog box where you set construction parameters.
4. Name, select data and describe the new molecule.
5. Verify and edit, where needed, the component fragments in the Goal Molecule Definition List.
6. Initiate molecule construction.

If construction is successful, your molecule is entered into the database and is opened in a new Molecule Display window.

The Molecule Editor is the tool for editing existing database molecules and for creating new basic molecules from scratch. For details, refer to page 295.


Construction Tips:

- Before you begin, open display windows for all of the molecules that are fragment sources. If you have several display windows open concurrently, the source molecule for your fragment must be the topmost window.
- It is sometimes convenient to work with two Molecule Display windows on display at the same time. Select **Window > Tile Vertical**. For each window, resize the Graphics Pane and molecule maps using the split bars, scroll bars and Zoom buttons to optimize viewing of the graphics. Click in a display window to activate it; an active window is designated by a dark blue Title Bar.

Describing Component Fragments in the Fragment Wizard

The Fragment Wizard guides you through several steps required to fully define molecule fragments suitable for construction or design. While in view, it allows you to continue to work in the Graphics Pane to select the fragment, while providing visual feedback and explanation of the actions.

To define a fragment using Fragment Wizard, open a display window for the molecule to be the source of a fragment.

Activate the Graphics Pane and press the **Add Fragment To Goal List** button () , or select **List > Add Fragment to Goal List**, opening the Fragment Wizard dialog box (Fig. 22.1):

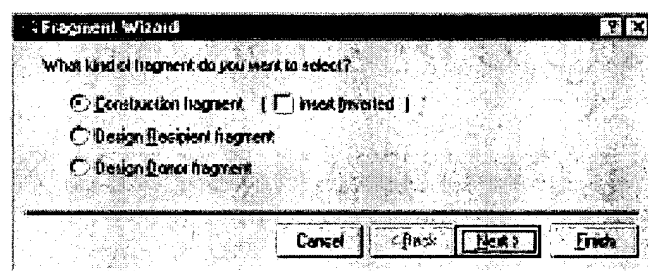


Fig. 22. 1 Fragment Wizard dialog box

The Fragment Wizard screen changes to display the entry fields and descriptions corresponding to the current step in the fragment definition process.

At any point in the process, you can choose to **Cancel**, go **Back** a screen, go to the **Next** screen, or **Finish** the process using the appropriate buttons. (**Finish** goes directly to the last step, skipping all the remaining steps, if any. Fragment Wizard goes through all the steps you skipped and supplies the missing information using default values and the coordinates of the current selection in the Display window.)

In the first "screen":

Select the fragment type (Fig. 22.1) and process: Construction fragment, Design Recipient fragment and Design Donor fragment. Since this chapter covers Construction, select **Construction Fragment**.

Design donor and design recipient fragments are discussed in Chapter 23.

When Construction fragment is selected, you can specify that the fragment should be inverted in the constructed molecule by checking the **Insert Inverted** box. Press the **Next** button to proceed.

In the second screen (Fig. 22.2):

Specify the 5' terminus of a new fragment. The options are:

- Set to a position on the molecule map (in one of the two following ways)
 1. Enter the coordinate in the text box or **CLICK** in the Graphics Pane of the display window. In the latter case, the option button will be selected automatically and the caret position is displayed in the position field of the dialog box and on the Status Bar. Use left or right arrows to reposition the caret.

Tip: If, when you click in the Graphics Pane, the entire molecule is selected (as indicated by the wire frame), click in the Sequence Pane to deselect, and begin again.

- 2. Specify the start of the molecule
To set the 5' terminus at the start of the molecule, select the Start of Molecule option in the dialog.
- Specify a restriction site.

Click on the site or its label in the Graphics Pane. The name of the restriction endonuclease and the position of the site are displayed in the Set to a Restriction Site box.

Note: If the Fragment Wizard screen limits your view of the molecule map, click on its title bar and drag it out of the way.

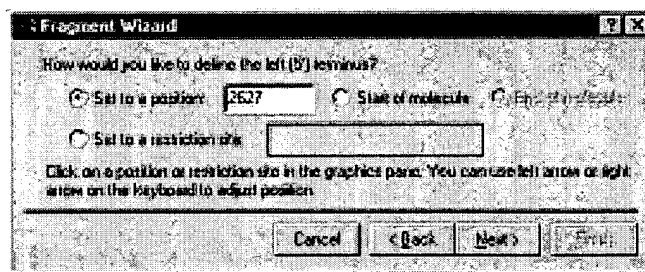


Fig. 22. 2 Specifying the 5' terminus of a new fragment

Press the **Next** button to proceed.

In the third screen (Fig. 22.3):

Specify the 3' terminus of the fragment. Options are:

- Specify a position on the molecule map (in one of the two following ways)
 1. Enter the position in the text box or SHIFT + CLICK in the Graphics Pane on a position, restriction site or label, automatically selecting this option. The 5'-3' selection is now indicated with a wireframe in the Graphics Pane and in the set selection box on the Status Bar. Reposition the 3' end using SHIFT + the left or right arrows.
 2. Specify the Start or End of the Molecule

The selection is indicated with a wireframe and on the Status Bar.

- Specify a restriction site.
SHIFT + CLICK on the site or its label in the Graphics Pane.

Tip: The SHIFT key must be held down for all actions in the Graphics Pane for setting the 3' end. If you neglect to do this, your actions will change the 5' terminus and Fragment Wizard will revert to screen 2.

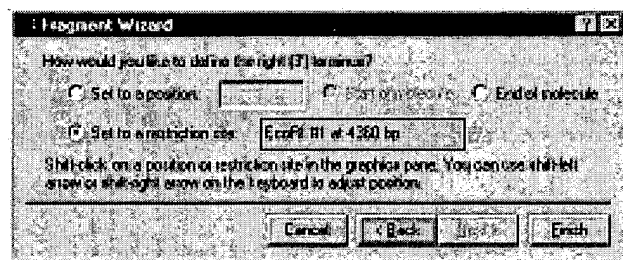


Fig. 22. 3 Specifying the 3' terminus of the fragment

Press the **Finish** button, completing the fragment definition.

Review the description of the selected fragment in the New Fragment message box (Fig. 22.4).

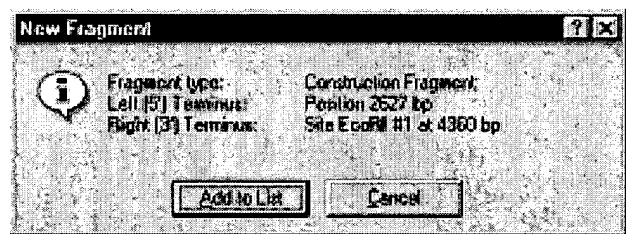


Fig. 22. 4 New Fragment message box


If you made a mistake, press **Cancel** to return to the Fragment Wizard. Otherwise, press the **Add to List** button, adding the fragment to the Goal Molecule Definition List and closing the Fragment Wizard.

Now go to the Molecule Display window for the molecule source of the second Construction fragment and define it using the Fragment Wizard in the manner just described. After reviewing your fragment as before in the New Fragment dialog box, enter it into the Goal Molecule Definition List.

The Goal Molecule Definition List (Goal List)

The Goal Molecule Definition List is a list of fragments that will be combined to create a new molecule.

You can add fragments to the Goal Molecule Definition List using the Fragment Wizard as just described or you can add them directly from the Construct dialog box.

To see the Goal Molecule Definition List, press the **Open Goal List** button () on the Main Toolbar or choose **List > Molecule Goal List**. At the bottom of the Lists dialog box (Fig. 22.5) that opens, the Fragments display lists the fragments currently on the Goal Molecule Definition List.

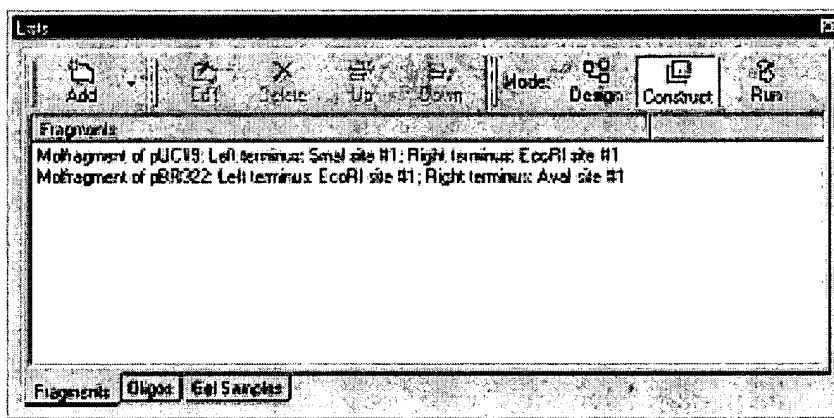



Fig. 22. 5 Lists dialog box

Constructing a New Molecule

The Construct & Design Molecule Dialog Boxes

In these dialog boxes, you can describe the new molecule to be constructed (or designed) and saved into the Vector NTI database. Also you can open it for an existing constructed or designed database molecule to reconstruct/redesign it, that is, after changing its component fragments.

To open the Construct or Design dialog box, first open the Lists dialog box from a Molecule

Display window by clicking the **Open Goal List** button () or selecting **Molecule > Create New > Using Construct/Design Procedure (DNA/RNA)** or selecting **List > Molecule Goal List**. Press the **Run** button on the Lists dialog box to display the Construct or Design Molecule dialog box. The dialog box is automatically loaded with the new molecule described through Goal Molecule Definition List.

Note: There are two operational modes—a mode for molecule Construction and mode for molecule Design. The appropriate mode is set automatically when you designate the component fragment type in Fragment Wizard. The title of the box “Construct Molecule” or “Design Molecule” reflects the mode. *(If this dialog box is opened before the method is designated, the box title is also Construct Molecule.)* Because Construction is the subject of this chapter, we will refer to it as Construct Molecule dialog box (Fig. 22.6).

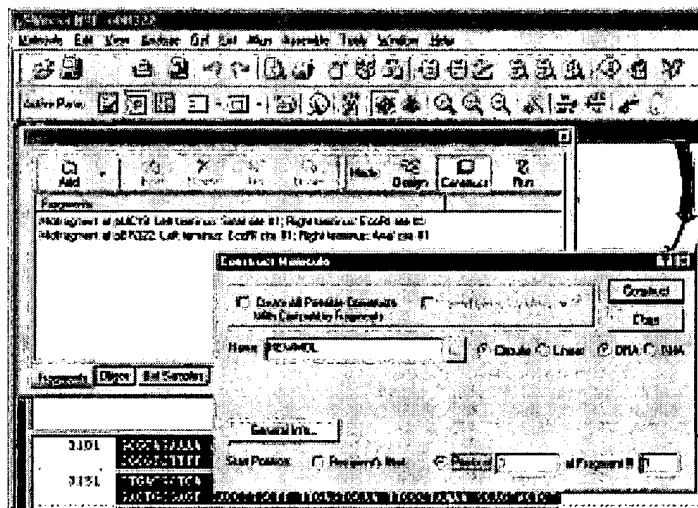


Fig. 22. 6 Construct Molecule dialog box

Enter or select the dialog box fields as follows:

- **Name** - enter a new name for the molecule to be constructed
- **Circular/Linear** - the form of the molecule.
- **DNA/RNA** - molecule type
- Click the **General Info** button to enter molecule description information:
 - **Description** - notes about the molecule
 - **Extra-Chromosome Replication** - transformation systems

- **Replicon Type** - molecule's replicon type
- **Keywords** - keywords useful for database search. See page 222.
- Click **OK** to return to the Construct dialog box.

*All of the **General Info** data will appear in the General Description folder when the molecule is loaded into a Display window.*

- **Recipient's Start** - start of the new molecule is as close as possible to the start of the "recipient" component fragment (the first fragment listed of the Component Fragments).
- **Position ...** specify a specific nucleotide of a specific fragment as the starting nucleotide of the new molecule. *(By default, the first nucleotide of the first fragment.)*

The Component Fragments Field

The Fragments field in the Lists dialog box contains the Goal Molecule Definition List. Constructed molecules consist of one or more component fragments that can be verified, entered, deleted, repositioned or edited in this field. In addition, you can add new fragments of five types, described below in the section on adding new fragments.

To perform an operation on a fragment, select it and press one of the following enabled buttons:

- **Edit:** This action can also be initiated by double-clicking the fragment. In the dialog box that opens, you can edit fragment information. The specific dialog box appears depends on the type of the fragment you have highlighted. Five fragment types (described below in the section on adding new fragments) each have their own Edit dialog box.
- **Delete:** The selected fragment disappears from the list.
- **Reposition a fragment:** To change the position of a fragment in the list, highlight the fragment you want to move, and press the **Up** or **Down** buttons to change its position in the list.

The molecule will be constructed from the fragments in the order shown, so changing the positions in the list changes the resulting molecule. The fragment listed first is always considered the "recipient" fragment.

Adding New Component Fragments

To add a new fragment to the list of component fragments, press the **Add** button above the Fragments field and choose the desired fragment type from the drop down list. In the corresponding Fragment Editor dialog box that opens, you can enter information about the new fragment:

- **Dummy:** A Dummy fragment can be created for a recombinant molecule as a last resort when a DNA fragment length is known but the exact sequence is not. Residues in

Dummy fragments are added as “nucleotides” only. *They will appear in a sequence as “N”.*

When you add or edit a Dummy fragment, the Dummy Fragment dialog box (Fig. 22.7) appears where you can enter the number of unsequenced nucleotides the fragment contains:

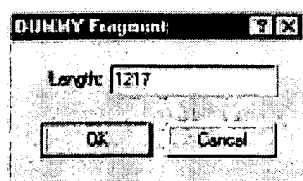


Fig. 22. 7 Dummy Fragment dialog box

Dummy fragments have blunt termini. A Dummy fragment must be inserted into a vector with compatible linkers or adaptors flanking the sequence. To add REN sites to ends of a Dummy fragment, see the Linker section below.

- **Sequence:** Sequence fragments are user-specified oligonucleotides. When you add or edit a Sequence fragment, the Sequence Fragment dialog box appears (Fig. 22.8):

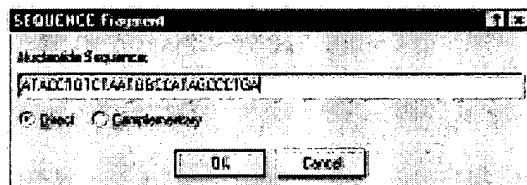


Fig. 22. 8 Sequence Fragment dialog box

In the Nucleotide Sequence field, enter an oligonucleotide up to 120 units long. With the radio buttons, indicate direct or complementary orientation.

Sequence fragments have blunt termini. A Sequence fragment must be inserted into a vector with compatible linkers flanking the sequence. To add REN sites to ends of a Sequence fragment, see the Linker section below.

- **Linker:** Linker fragments are single restriction sites that can have short flanking sequences. They are used to link together longer fragments in Construction or Design. When you add or edit a Linker fragment, the Linker Fragment dialog box (Fig. 22.9) appears:

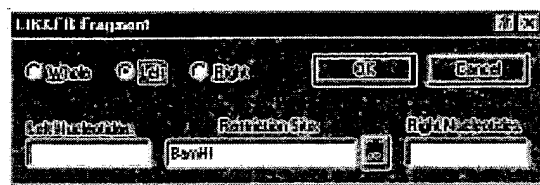



Fig. 22. 9 Linker Fragment dialog box

In the Restriction Site field, enter the name of the restriction endonuclease being used or press the **Browse** button () to select from a list of RENs in the database.

In the Left Nucleotides and Right Nucleotides fields, you can enter nucleotide sequences flanking the restriction site.

Once the restriction site is in place, select the radio button that indicates whether the linker should be the whole restriction site or whether it will attach at the left end or the right end of another sequence or dummy fragment.

If you click **whole**, then both ends of the Linker are blunt. If you click **Left**, the 5' end is blunt and the 3' end is the restriction site. If you click **Right**, the 3' end is blunt and the 5' end is the restriction site.

Examples:

Left Linker **HindIII** A TTCCA Right Linker **EcoRI** AATTC G

If you want restriction site Linkers to be attached on each end of a sequence to be cloned, you must generate a right and a left Linker. In the following example, the Left Linker will ligate to the 5' end of the digested recipient fragment and the Right Linker will ligate to its 3' end.

Right Linker **EcoRI** AATTC G Dummy Fragment TTCCA Left Linker **HindIII** A

Construction Tip: To add linkers at each end of a Dummy or Sequence fragment, they should be listed in the Component Fragment list in the following order:

- Recipient Fragment
- Linker (Left Linker)

- Dummy or Sequence
- Linker (Right Linker)
- **Adaptor:** Adaptor fragments are like linkers except that you enter or edit nucleotides manually in the Adaptor Fragment dialog box (Fig. 22.10). Adaptors are often special-ordered from a synthesizer vendor, as they tend to be linkers that are “adapted” to the given experiment/need at hand.

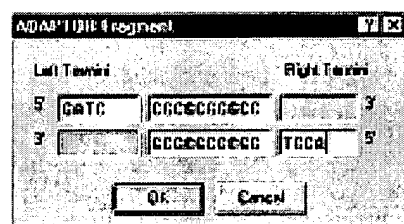


Fig. 22.10 Adaptor Fragment dialog box

In Vector NTI, an Adaptor consists of a 5' or 3' left terminus, a central double-stranded part, and a 5' or 3' right terminus, although any part of an Adaptor fragment is optional. Enter these segments in the appropriate fields of the dialog box. Although the example in Fig. 22.10 has staggered ends, they could be blunt.

- **Add Fragment:**

There are three choices available from the Add Fragment selection, adding a fragment from the molecule that is currently in focus in the VNTI workspace, adding a fragment of a molecule in the VNTI database or adding a fragment using the Fragment Editor. The first two choices will open the appropriate molecule and launch the Fragment Wizard. The third choice will launch the Fragment Editor.

Note: Although you can add molecule fragments using the Fragment Editor, it is much more convenient to add them either in advance using the Fragment Wizard—the tool specifically designed for visual selection of molecule fragments from Molecule Display windows (See page 351) or via the first two choices on the Add Fragment list, which also use the Fragment Wizard.

Fragments of existing molecules are the key part of most constructed molecules. Press the **Add** button and select the **Add Fragment > with Fragment Editor** choice, or select a component fragment in the list and click the **Edit** button to open the Fragment of Molecule dialog box (Fig. 22.11) where you add or edit such a fragment:

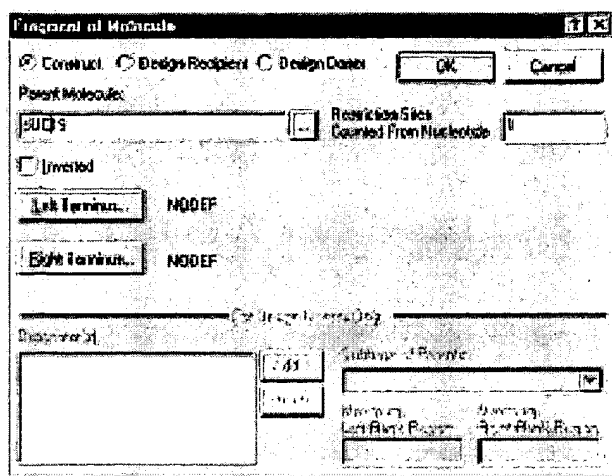



Fig. 22. 11 Fragment of Molecule dialog box

Options in this dialog box are summarized as follows:

- **Construct/Design Recipient/Design Donor:** molecule fragment type
- **Parent Molecule:** Enter the name of the source molecule for the fragment to be added, or press the **Browse** button () to select a molecule from the Vector NTI database.
- **Inverted:** Check to insert the fragment in the inverted orientation.
- **Restriction Sites Counted From Nucleotide:** Enter the position in the parent molecule from which restriction site occurrences should be counted. This field is important when this fragment is taken from a selection display window, where the numbering of sites in the selection display window may not correspond to the numbering of sites in the molecule as a whole. Usually the value of this field is 1.
- **Termini:** To describe a fragment from an existing molecule, you must describe its left and right termini. Brief descriptions of the termini are displayed next to the corresponding buttons. Click each of the <...Terminus> buttons to define the termini.

Editing the Termini of Fragment of Molecule-type Component Fragments

Press the **Left Terminus** or **Right Terminus** buttons in to open the Terminus Editor dialog box (Fig. 22.12).

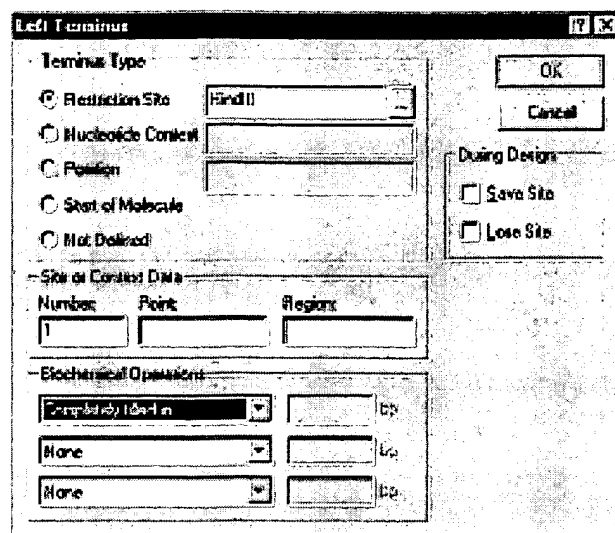



Fig. 22. 12 Terminus Editor dialog box

The Left (and Right) Terminus dialog box options are summarized as follows:

Terminus Type:

- **Restriction site:** If the terminus is a restriction site, enter its name in the text box, or press the **Browse** button () to select from a list of the RENs in the database.

The restriction site chosen here determines the form of the terminus (blunt, 5', 3'). The terminus can be further modified by biochemical operations, as described below.
- **Nucleotide context:** If the terminus is a nucleotide context (oligonucleotide), enter the sequence in the text box.

This type of terminus is blunt.
- **Position:** If the terminus is a position in the molecule's nucleotide sequence, enter its position in the text box.

This type of terminus is blunt.
- **Start / End of Molecule:** If the terminus is the start or end of the parent molecule, click the appropriate radio button.

This type of terminus is blunt.
- **Not Defined (NODEF):** Click this button if the terminus is undefined.

A fragment with one or more NODEF termini can only be used for automatic molecule Design, discussed in the next chapter.

Sit or Context Data

If the terminus is a restriction site or nucleotide context, these fields are enabled where you are required to indicate where on the parent molecule the site or context can be found.

- Enter an integer value in the Number field to use that occurrence of the site or context on the parent molecule.

In place of a number, you can enter an area of the molecule within which the site or context occurs only once:

- Enter a value in the Point and Region fields to look for the site or context within (Region) nucleotides of the (Point) nucleotide of the parent molecule.

Vector NTI uses the Point and Region fields only if the Number field is empty.

Biochemical Operations

Termini of Restriction Site type can be treated with up to three consecutive biochemical operations, performed in the sequence listed in the Terminus Editor dialog box. The operations are performed in the order in which they are listed.

These operations from the lists in the Biochemical Operations drop-down menus have the following effects:

- **None** leaves the terminus untreated.
- **Completely filled in** fills in cohesive fragment termini to make them blunt.
- **Incompletely filled in** fills in cohesive fragment termini to within a few nucleotides of being blunt.
- **S1-treated** cuts cohesive termini down to blunt.
- **Bal31 treated** cuts off a given number of nucleotides from both strands.
- **3'-5' exonuclease treated** cuts off a given number of nucleotides in the 3'-5' direction.
- **5'-3' exonuclease treated** cuts off a given number of nucleotides in the 5'-3' direction.

Click **OK** to return to the Fragment Molecule dialog box (Fig. 27.6).

The For Design Only subsection of this dialog box is enabled only in Design mode and is described in Chapter 23.

Click **OK** to return to the Lists dialog box. Press the **Run** button to launch the Construct Molecule dialog box.

The Control Fields and Commands

In Construction mode, the following mode is enabled: **Create All Possible Constructs...**

When checked, the system creates all possible constructions (up to 24) that can be obtained from the molecule component fragments. *This option allows Vector NTI to include even the parasitic constructs (inserts in the wrong orientation) in the reaction mix.*

The **Open Display Windows for All** field is active only when the **Create All Possible Constructs** box is checked or the specified number of design output options is more than one. If this box is checked, and more than one molecule is created during the construction or design process, Molecule Display windows are opened for each new molecule immediately after completing the procedure. If this box remains unchecked, a new Molecule Display window opens automatically for just the molecule with the name specified in the Name field.

Note: In Design, display windows are not created for intermediate molecules, but only for the final option(s).

Once all parameters are set in the Construct Molecule dialog box, press the **Construct** button to initiate the process.

Naming of Construction Recombinants

If you start a construction task for a goal molecule with a name that causes a conflict with an existing set of construction options, Vector NTI informs you of the conflict and asks permission to delete the existing options. To avoid this, choose a different name for the new goal molecule or rename old construction options.

For additional constructs, Vector NTI creates names on the basis of the name of the “main” constructed molecule. If the name of the molecule is <MOLNAME>, the name of the first additional construct will be <MOLNAME>_#1, the name of the second additional construct will be <MOLNAME>_#2, etc.

As Construction proceeds, Vector NTI creates a special subbase for the molecules, named by the program on the basis of the name of the “main” constructed molecule. If the name of the molecule is <MOLNAME>, then the name of the subbase containing additional constructs will be <MOLNAME>_\$_Construction Options.

Chapter 23 Molecule Design

Introduction

This chapter describes the creation of DNA molecules through Design.

In Design, you can describe the list of fragments in very general or very specific terms. You can also describe preferences for techniques used in creating the molecule, such as how fragments may be isolated from their parent molecules, how termini may be modified, etc. In contrast to the Construction technique described in Chapter 22, in Design Vector NTI does the rest, taking advantage of the best possible restriction sites and genetic engineering techniques to engineer the molecule.

This chapter describes:

- The overall procedure for designing a DNA molecule
- How to describe a goal molecule using Display windows, Fragment Wizard, and the Molecule Editor
- How to start the design process
- How to set your preferences for design techniques
- What Vector NTI actually does during the design process, and how you can control the process
- Discussion of the design plans Vector NTI generates.

Creating a New DNA Molecule Using Design

For creating a molecule by Design, the Molecule Display window must be in Molecule Edit mode, the default mode when the window is opened.

The process of creating a new molecule with Vector NTI's Design capabilities generally follows these steps in an active Graphics Pane:

1. Describe your goal molecule by defining its recipient and donor fragments and placing them in the Goal Molecule Definition List in the proper order using Fragment Wizard.
2. Open the Lists dialog box, loaded with the component fragments for your Goal Molecule.
3. Open the Design Molecule dialog box, enter general information for the new molecule and initiate the Design.
4. When the Design Parameters dialog box appears, set the appropriate parameters and your design preferences.

5. Start the design process. Vector NTI searches for optimum Design options. If Design is successful, your molecule is entered into the database and opens in its new Molecule Display window.
6. In the Text Pane of the display window containing the goal molecule, open the Design Description folder to inspect the design plan Vector NTI has generated.

If you are not satisfied with the design plan, you can choose to redesign the molecule by changing the description of the goal molecule or using different parameters.

Describing the Goal Molecule


In Design, you must define one *recipient fragment*, listed first in the Goal Molecule Definition List, and one or more *donor fragments*.

The simplest way to define fragments for Design is to use the Fragment Wizard and the Graphics Pane to visually define fragments. You can then use the Fragment Editor to fine-tune fragment descriptions. Fragments can be defined by hand in the Design Molecule dialog box, but it is much more tedious and much less effective than using Fragment Wizard. Refer to Chapter 22 for a detailed description of Fragment Wizard. Here we will describe how to use it to define design recipient and design donor fragments.

Defining the Recipient Using Fragment Wizard

- The recipient fragment of the goal molecule remains unaffected during the Design process. All nucleotides within the recipient fragment you defined are included in the goal molecule. This means that any polylinker sites used for cloning must be outside the recipient fragment.
- The recipient must be listed as the first fragment in the Goal Molecule Definition List. This will be covered later in the chapter.
- The recipient must come from a circular molecule stored in the Vector NTI database.

To define a recipient fragment, open the Display window for the recipient molecule. If you have several display windows open concurrently, you can display the source molecules concurrently by selecting **Window > Tile Vertically** or make sure the source molecule for your fragment is the topmost window. Activate its Graphics Pane and press the **Add**

Fragment To Goal List button () or select **List > Add Fragment to Goal List**, opening The Fragment Wizard dialog box (Fig. 23.1).

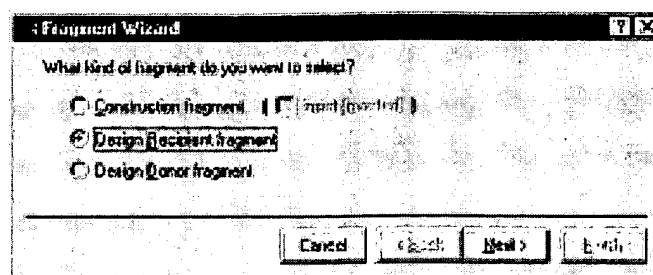


Fig. 23. 1 Fragment Wizard dialog box

The Fragment Wizard dialog box content changes to display the entry fields and descriptions corresponding to the current step in the fragment definition process.

At any point in the process, you can choose to **Cancel**, go **Back** a screen, go to the **Next** screen, or **Finish** the process using the appropriate buttons. (**Finish** goes directly to the last step, skipping all the remaining steps, if any. Fragment Wizard goes through all the steps you skipped and supplies the missing information using default values and the coordinates of the current selection in the Display window.)

In the first "screen":

Select the kind of fragment you wish to define from three choices: Since this chapter covers Design, choose the **Design Recipient Fragment** option and press the **Next** button to go to the second screen of Fragment Wizard:

In the second screen (Fig. 23.2):

To specify the 5' terminus of the fragment, select one of the following options. *Vector NTI will look for good cloning sites in the region upstream from the position you specify.*

- Specify a position on the molecule map (in one of the two following ways)
 1. Enter the coordinate in the text box or click in the Graphics Pane of the display window. In the latter case, the Set to a Position button will be selected automatically and the caret position is displayed in the position field of the dialog box (and on the Status Bar). To reposition the caret, use the right and left arrow keys.

Tip: If, when you click in the Graphics Pane, the entire molecule is selected (as indicated by the wire frame), click in the Sequence Pane to deselect, and begin again.
 2. Specify the start of the molecule by selecting the Start of Molecule option in the dialog box.
- Specify a restriction site.

To set the terminus to a restriction site, click on the site or its label in the Graphics Pane. *If selected, Vector NTI must use that site in cloning. For Design, you may be setting an unrealistic limitation. If the site is not convenient, for example, requiring complicated partial digestion, the cloning may be very difficult.*

Note: If the Fragment Wizard screen limits your view of the molecule map, left click on Wizard's blue title bar, and drag it out of the way.

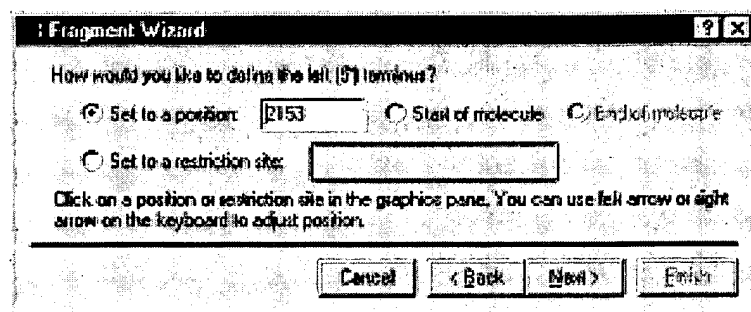


Fig. 23. 2 Specifying the 5' terminus of the fragment

When the 5' terminus is set, press the **Next** button to proceed.

In the third screen (Fig. 23.3):

This screen appears only if you specified that the 5' terminus should be set to a palindromic restriction site. If that was not your choice, move on to the description of the fourth screen.

You are asked to specify whether the site should be saved or lost in the designed molecule. Your options are: **Save Site**, **Lose Site** or **Don't Care**. If you do not specify that site is to be saved or lost, its fate will be unpredictable. Choose the desired option and press the **Next** button to advance to the next screen of the dialog box.

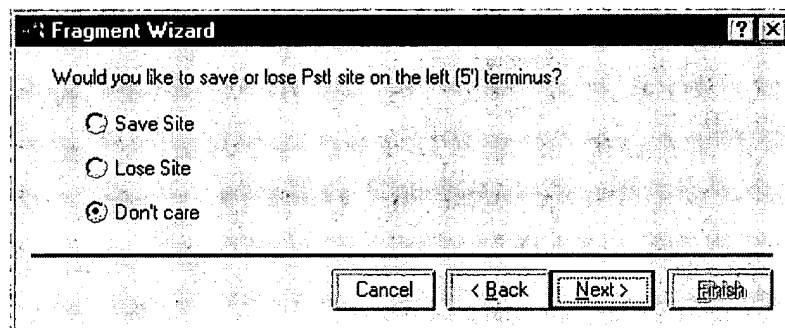


Fig. 23. 3 Specifying whether the site should be saved or lost in the designed molecule

In the fourth screen:

Specify the 3' terminus of the fragment. Vector NTI will look for good cloning sites in the region following the position you specify. Options are:

- Specify a position on the molecule map (in one of the two following ways)
 1. Enter the position into the text box or SHIFT + CLICK in the Graphics Pane and adjust the caret position using shift-arrow key combinations described in Chapter 3.
 2. Specify the end of the molecule

- Specify a restriction site.

SHIFT + CLICK on the site or its label in the Graphics Pane. If selected, Vector NTI must use that site in cloning. *For Design, you may be setting an unrealistic limitation. If the site is not convenient, for example, requiring complicated partial digestion, the cloning may be very difficult.*

Tip: The shift key must be held down for all actions in the Graphics Pane for setting the 3' end. If you neglect to do this, your actions will change the 5' terminus and Fragment Wizard will revert to screen 2.

If you specified that the 3' terminus is set at a palindromic restriction site, you can go to the next screen and specify that the site should be saved or lost in the designed molecule. Choose the desired option and press the **Finish** button to complete the definition of the fragment.

If you did not specify a palindromic restriction site, the **Next** button is disabled; press the **Finish** button, signifying completion of fragment definition.

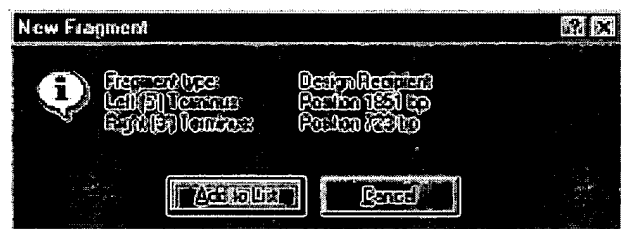


Fig. 23. 4 Adding the fragment to the Goal Molecule Definition List

Review the description of the selected fragment in the New Fragment message box. If you made a mistake, press **Cancel** button to return to the Fragment Wizard. Otherwise, press the **Add to List** button (Fig. 23.4), adding the fragment to the Goal Molecule Definition List and closing the Fragment Wizard.

Notes about Design:

- If you later edit the recipient fragment in the List/Design Molecule dialog box, any biochemical operations you order to be performed on the termini of the recipient

fragment will be ignored. During the Design process, Vector NTI chooses all methods of terminus modification itself.


- The recipient fragment always has direct orientation. The Inverted box in the recipient's Fragment Editor dialog is disabled during the Design process.

Defining the Donors Using Fragment Wizard

Donors are fragments that Vector NTI inserts into the recipient fragment (vector) to produce the goal molecule. Donor fragments in Design must carry functional signals into the recipient.

When performing Design, Vector NTI requires all fragments except the first in the Goal Molecule Definition List to be donor fragments. Donor fragments can come from circular or linear molecules stored in the database.

To define a donor fragment, open the display window for the donor molecule. *(If you have several display windows open concurrently, the source molecule for your fragment should be the topmost window.)* Activate its Graphics Pane and press the **Add Fragment To Goal**

List button  on the Window toolbar or select **List > Add Fragment to Goal List**, opening The Fragment Wizard dialog box.

In the first screen:

Select the Design Donor Fragment option and press the **Next** button to go to the second screen of the Fragment Wizard.

In the second screen (Fig. 23.5):

- To select the desired functional signals to be carried on the donor fragment into the recipient, click on the signal or its label in the Graphics Pane. To select more than one signal, hold down the SHIFT + CLICK. *Note: If the Fragment Wizard screen limits your view of the molecule map, left click on Wizard's blue title bar, and drag it out of the way.*
- To deselect a selected signal, hold down SHIFT + CLICK again.

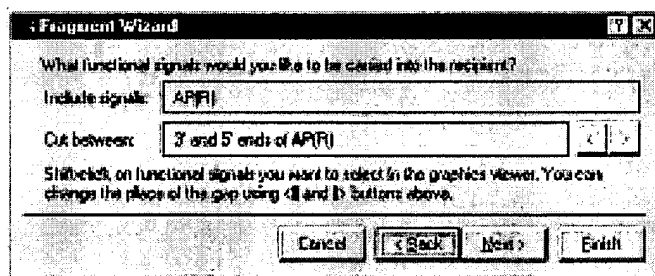


Fig. 23. 5 Selecting the desired functional signals to be carried on the donor fragment into the recipient

The names of the signals you selected are displayed in the Fragment Wizard dialog box.

If the donor molecule is circular and you have chosen more than one signal, Fragment Wizard lets you choose the place of the “gap” between signals where Vector NTI will look for a convenient place to cut the donor molecule. To move the gap clockwise, press the right arrow button on the Fragment Wizard; counterclockwise, press the left arrow button. To illustrate this, select more than one signal and experiment. When the desired signals are selected, press the **Next** button to go to the next screen.

In the third screen (Fig. 23.6):

The next screen for specifying the 5' terminus for design offers two selections:

- **Leave Terminus Undefined** if you want Vector NTI to choose restriction sites for you
- **Use Specific Site** to use a restriction site to cut the signals from the donor molecule. Fragment Wizard prompts you to hold down the SHIFT key and click on the restriction site or its label in the Graphics Pane. The site label is selected and the name of the site and its position are shown in the Fragment Wizard dialog box. *If selected in this way, Vector NTI is forced to use that site in cloning. If the site is not convenient, for example, requiring complicated partial digestion, you will be responsible for the difficulty of the cloning situation.*

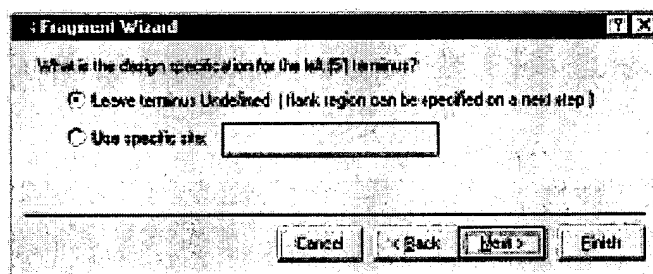


Fig. 23. 6 Specifying the 5' terminus for design

Press the **Next** button to go to the next screen.

In the fourth screen (Fig. 23.7):

If you have chosen the **Use Specific Site** option, this screen lets you specify the 3' terminus for design. The required actions are the same as for the 5' terminus screen described above.

If you have chosen the **Leave Terminus Undefined** option, this screen asks you to specify the maximum size of the 5' flank region (if you came from the 5' terminus definition screen) or 3' flank region.

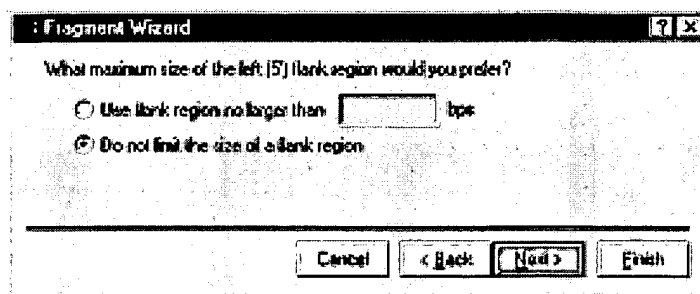


Fig. 23. 7 Specifying the 3' terminus for design, or specifying the maximum size of the 5' flank region


- **Use Flank Region No Larger Than ... bps** limits the region within which cloning sites may be sought (thus limiting the size of the resulting flank region). At the prompt, enter either the maximum length of the flank region in the dialog's text box or move the appropriate end of the selection in the Graphics Pane into the gap beyond the edges of the selected functional signals. In this case, the selection in the Graphics Pane shows a maximum possible flank region. You can use all available techniques for extending a selection in the Graphics Pane: drag the appropriate end of the selection, SHIFT-click on the desired position, etc. For details on Selection Techniques, refer to Chapter 3.
- **Do Not Limit the Size of a Flank Region** allows Vector NTI to search all available space for restriction sites.

When you are finished with the description of both termini of the donor fragment, the **Next** button becomes disabled. Press the **Finish** button to complete the definition of the donor fragment.

The New Fragment message box is displayed with the description of the selected donor fragment. If you made a mistake, press the **Cancel** button to return to the Fragment Wizard dialog. Otherwise, press the **Add to List** button. The fragment is added to the Goal Molecule Definition List and the Fragment Wizard dialog box is closed.

The Goal Molecule Definition List (Goal List)

The Goal Molecule Definition List (Fig. 23.8) (in the **Fragments** tab at the bottom of the Lists dialog box) is a list of fragments to be combined to create a new molecule. In addition to adding fragments to the Goal Molecule Definition List using the Fragment Wizard, you can add them directly from the Lists dialog box.

To see the Goal Molecule Definition List, press the **Show Goal List** button () or choose **List > Molecule Goal List**. In the Lists dialog box (**Fragments** tab) that opens, the Fragments display box lists the fragments currently in the Goal Molecule Definition List.

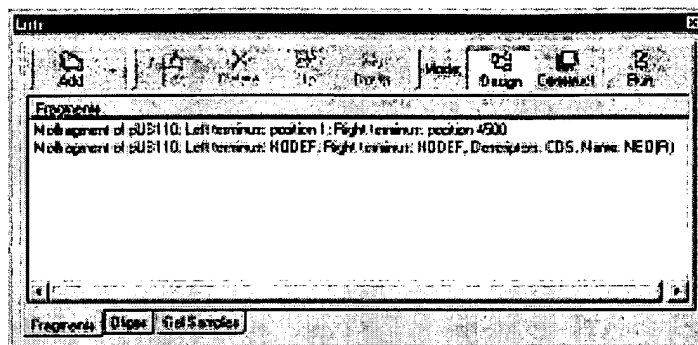


Fig. 23. 8 Goal Molecule Definition List

Note: The first fragment listed is considered the “recipient fragment.” If a fragment is listed in incorrect order, select it and click the **Up** or **Down** buttons to reposition it.

Starting the Design

Although the goal molecule component fragments are ready for processing by Vector NTI’s Design mode, remember that VNTI decides on the optimum Design fragment termini and cloning strategies.

To begin the design of your new molecule, open the Lists dialog box by clicking on the **Open Goal List** button or selecting **List > Molecule Goal List**.

Open the Design Molecule dialog box by pressing the **Run** button on the Lists dialog box.

Note: The Construct/Design Molecule dialog box has two operational modes—a mode for molecule construction and mode for molecule design. Because you have defined fragments in this chapter for Design, in this mode, the dialog box title is the Design Molecule. See Chapter 22 for details on the Construct Mode.

Enter or select the dialog box fields as follows:

- **Name**
- **Circular/Linear** - the form of the molecule.
- **DNA/RNA** - molecule type
- Click the **General Info** button to enter molecule description information:
 - **Description** - notes about the molecule
 - **Extra-Chromosome Replication** - transformation systems
 - **Replicon Type** - molecule’s replicon type
 - **Keywords** - keywords useful for database search. Refer to page 222 for details.

- Click **OK** to return to the Design dialog box.

*All of the **General Info** data will appear in the General Description folder when the molecule is loaded into a Display window.*

- **Recipient's Start** - start of the new molecule is as close as possible to the start of the "recipient" component fragment (the first fragment listed of the Component Fragments).
- **Position ...** - specify a specific nucleotide of a specific fragment as the starting nucleotide of the new molecule. (*By default, the first nucleotide of the first fragment.*)

The Component Fragments Field

Designed molecules consist of one or more component fragments. Use the Fragments field in the Lists dialog box to verify or enter this information.

You may edit, delete, and change the order of the fragments in the Component Fragments field, and you may add new fragments of five types. These operations are described in Chapter 22.

It is essential in molecule design that the recipient fragment is first in the Fragments list, followed by the donor fragments. If you need to change this order, highlight the fragment(s) to be moved and press the **Up** or **Down** buttons until they are in the correct order.

Defining Design Fragments in Fragment Editor

An alternative to using Fragment Wizard for defining design fragments is to enter them manually into the **Fragments** tab of the Lists dialog. To do this, press the **Add** button and choose **Add Fragment > with Fragment Editor** to open the Fragment of Molecule dialog box (Fig. 23.9).

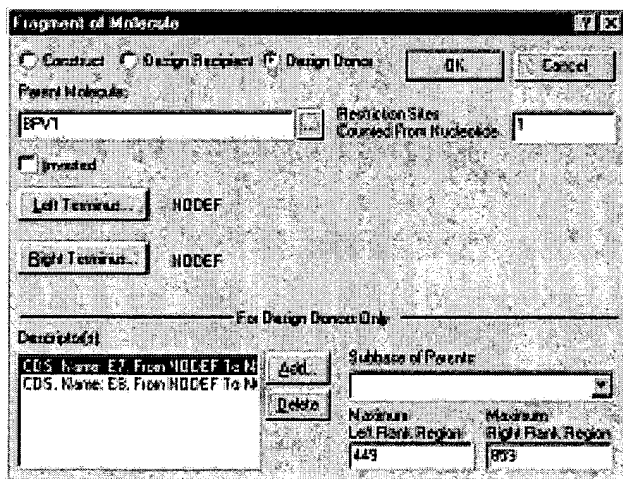


Fig. 23. 9 Fragment of Molecule dialog box

Options in the Fragment Editor dialog box are summarized as follows:

- **Donor recipient/donor fragment**
- A donor fragment created with a Display window always has a defined **parent molecule name**
- Change the orientation of a donor fragment by checking the **Inverted** box.
- **Left/Right Terminus** buttons open the Edit Terminus dialog box (Fig. 22.12). The way you defined the termini initially governs which sections are enabled in this dialog box. For more information on modifying termini, refer to Chapter 22.
- In Design, all donor fragments carry functional signals called descriptors into the recipient. To add or remove descriptors from a donor fragment, use the **For Design Donors Only** section of this dialog box. To add a descriptor, press the **Add** button, opening the Fragment Descriptor dialog box (Fig. 23.10):

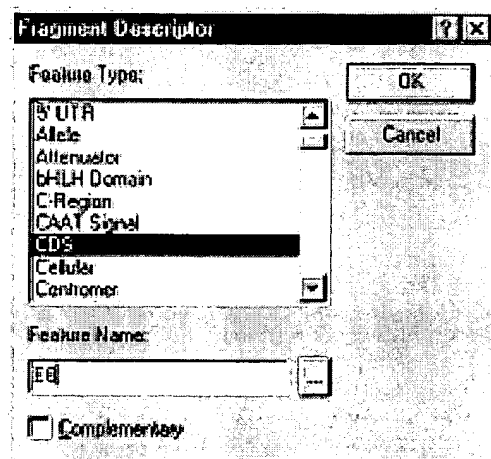


Fig. 23. 10 Fragment Descriptor dialog box

To define a descriptor for the donor fragment, choose the type of signal and enter the name of the signal, if necessary. If the signal is on the complementary strand, check the **Complementary** box. (If you do not enter a signal name, the descriptor is added to the donor fragment and is treated as any signal of the specified type.)

Note: The Fragment Descriptor dialog box is the only way to add a descriptor containing any signal of a specified type. Such a descriptor cannot be added from a display window.

Click **OK** to return to the Fragment of Molecule dialog box (Fig. 23.9).

- To remove a descriptor from a donor fragment's description, highlight it in the **Descriptor(s)** box and press the **Delete** button.

- If after leaving the Display window you would like to add flank regions to a donor fragment, or change flank regions set through a Display window, you can use the **Maximum Flank Region** boxes.

Subbase of Parents – If you prefer to simply identify the feature to be added to the new molecule, you can let Vector NTI decide which specific molecule of the subbase is used as the source for the functional signal. In the Subbase of Parents drop-down menu, select the subbase where the parent molecules might be sought.

After entering and verifying the data in the Lists dialog box, you are ready to proceed with the design process. Press the **Run** button to launch the Design Molecule dialog box and then press the **Design** button.

Vector NTI performs a preliminary save of the current molecule state. The molecule's name, general information, fragment list, etc. are saved and can be recalled. The system then performs a preliminary analysis of the design possibilities, and opens the Design Parameters dialog box, described below.

If you start a design for a goal molecule with a name that causes a conflict with an existing set of design options, Vector NTI informs you of the conflict and ask permission to delete the existing options. If you do not want to do this, choose a different name for the new goal molecule or rename old design options.

Design Parameters

After the Design is initiated, in the interim dialog box that opens, select a subbase for storing Design results. The Design Parameters dialog box (Fig. 23.11) then opens where you can set the Design parameters the program uses to design the molecule.

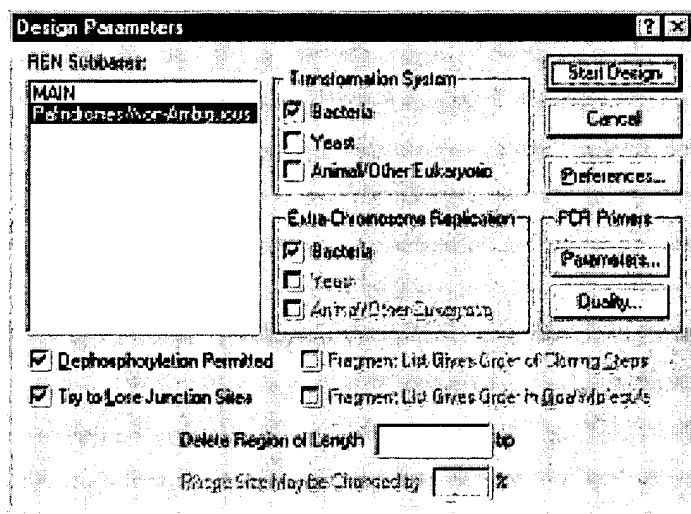


Fig. 23. 11 Design Parameters dialog box

Options in the Design Parameters dialog box are summarized as follows:

- **REN Subbases** displays the list of restriction endonucleases that Vector NTI will consider in the current Design task.
- **Transformation System(s)** and **Extra-Chromosome Replication** specifies the capabilities for the current Design process.
- **Dephosphorylation Permitted** allows dephosphorylation to be used as a preselection method.
- **Try to Lose Junction Sites** directs the loss of junction sites if possible without increasing cloning complexity. Lost sites are then used for efficient preselection. This box is a request, not a command, and Vector NTI may save the site if that results in a more convenient design option. *If, in the Fragment Wizard, you specified that sites be saved or lost in the description of the recipient, they will automatically be saved or lost regardless of whether this box is checked.*
- **Fragment List Gives Order of Cloning Steps** inserts the first donor into the recipient in the first cloning step, the second donor in the second cloning step, etc. *If this box is unchecked, the order of cloning steps is based on the most convenient restriction sites.*
- **Fragment List Gives Order in Goal Molecule** places the donor fragments in the goal molecule in the same order in which they are listed in the goal molecule description. *If this box is unchecked, the placement order is based on the most convenient restriction sites.*
- **Delete Region** specifies the number of nucleotides to be deleted from the recipient molecule during cloning. *This may be disabled, depending on how the fragments were defined.*
- **Phage Size May Be Changed By** is enabled if the recipient molecule is a phage, which may lose its functionality due to length changes. If you want to preserve phage functionality in the goal molecule, it may be useful to prevent the goal molecule from being much different in length than the recipient molecule. Enter the maximum change in length (%) for phages in the field. *If this field is enabled and specified, then the lengths of the goal molecule and all intermediate recombinants produced by the Design process are kept within the specified length limits.*
- **Preferences** opens the Design Preferences dialog box. See next section.
- **PCR Primers**

Vector NTI can use PCR amplification to isolate donor fragments. *The system also will propose primers for PCR amplification of cloned fragments in all molecules produced by the Design process, including the goal molecule.*

- Press the **Parameters** or **Quality** buttons under PCR Primers to set the desired parameters or Quality Specifics of PCR primers. Refer to Chapter 21 for the details of the dialog boxes that open with these commands.

The primers used will satisfy the conditions set by the user in the PCR Analysis dialog box.

When all design parameters are set as you desire, press the **Start Design** button.

D sign Pref renc s

Although much of the Design process is decided by Vector NTI, you can select preferred techniques for designing the molecules. Press the **Preferences** button in the Design Parameters dialog box (Fig. 23.11) to open the Design Preferences dialog box (Fig. 23.12):

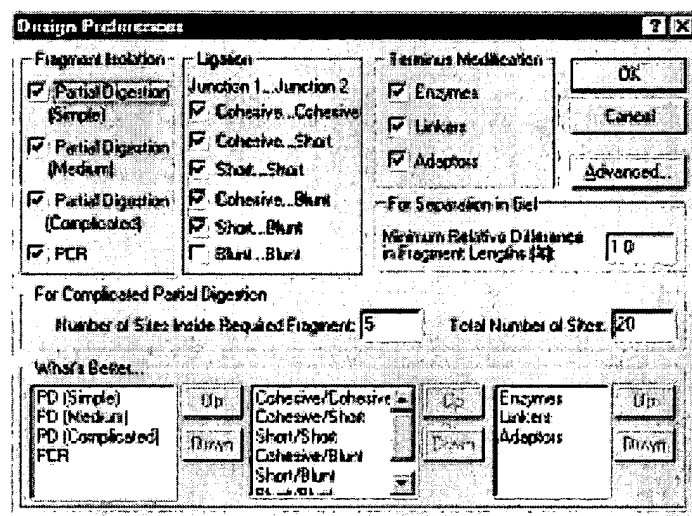


Fig. 23. 12 Design Preferences dialog box

In this dialog box, you can set preferences in the categories of fragment isolation, fragment ligation, and terminus modification.

Depending upon your Design results, you may find it useful to have Vector NTI design the same goal molecule several times, changing the design preferences each time. This gives you several alternatives for constructing your molecule.

The parameters for this dialog box are as follows:

- **Fragment isolation:** Vector NTI isolates fragments for cloning using convenient restriction sites whenever possible. If no convenient restriction sites are present, partial digestion or PCR can be used to isolate fragments. Check the isolation methods you will permit:
 - **Partial Digestion (Simple):** Partial Digestion with 1 site inside required fragment and 3 sites total.

- **Partial Digestion (Medium):** Partial Digestion with 1 site inside required fragment and not more than 6 sites total.
- **Partial Digestion (Complicated):** Partial Digestion with more than 1 identical site inside required fragment.
- **PCR**
- **Ligation:** Check the junction types you will permit Vector NTI to use in designing molecules. **Cohesive...Cohesive** junction means both ligated fragments have two cohesive ends, each longer than 1 nucleotide; **Cohesive...Short junction** means both ligated fragments have one cohesive and one short (exactly 1 nucleotide) end, etc.
- **Terminus modification:** Check the methods you will permit to make termini compatible.

Other Preferences

- **Separation in Gel:** Specify the minimum relative difference in length (%) for separating fragments in gel.
- **Complicated Partial Digestion:** Specify the maximum number of restriction sites inside the required fragment and the total number of sites on the molecule that are acceptable for fragment isolation using complicated partial digestion.

Priorities

What's Better – rate priorities for the techniques and ligation types by moving them in the corresponding lists. To reposition any item, select it and press the **Up** and **Down** buttons. Items near the top of the lists have higher priority than items lower in the lists.

Advanced Design Preferences

Press the **Advanced** button in the Design Preferences dialog box to open the Internal Design Parameters dialog box (Fig. 23.13) to fine-tune Vector NTI's heuristic search for molecule design options. *Default settings are given in italics.*

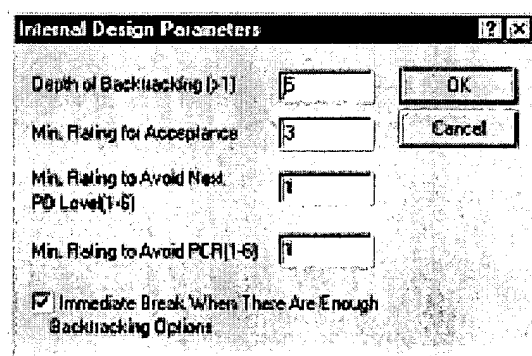


Fig. 23. 13 Internal Design Parameters dialog box

- **Depth of Backtracking:** Specify the number of steps Vector NTI should retrace in its search algorithm to design a molecule if it hits a dead-end in finding a molecule according to the defined parameters. 5
- **Min. Rating for Acceptance:** Specify the minimum acceptable rating to immediately accept an option and end the current search step. Vector NTI rates design options on an integer scale of 1 to 8. The higher the number, the longer Vector NTI continues searching for a better construct according to the defined parameters. 3
- **Min. Rating to Avoid Next PD Level:** If Vector NTI cannot achieve a minimum rating for acceptance (see previous point), it may try the next more complicated level of PD (partial digestion.) For instance, if you have listed Simple Digest first (under What's Better in Fig. 23.12), then Medium and then Complicated as partial digest preferences, Vector NTI tries to use the Simple Digest first. If it cannot reach the minimum rating for acceptance as specified, then it will use the next level of partial digestion, (medium) and so forth. Specify the rating below which the system will try more complicated partial digestion. 1
- **Min. Rating to Avoid PCR:** If Vector NTI cannot achieve a minimum rating for acceptance (see second point above), it will try to apply PCR. Specify the rating below which the system will try PCR. 1
- **Immediate Break When There Are Enough Backtracking Options:** If checked, Vector NTI stops searching for further solutions once it finds enough alternatives for backtracking. Checking this option can result in faster searches. Default *Yes*. *When this option is checked, the optimum design option will usually be among those found, but sometimes the system may find something better if permitted further search*

Click **OK** twice to return to the Design Parameters dialog box.

Note: InforMax recommends that you not change these parameters until you have developed extensive experience with Vector NTI's Design capabilities.

The Design Process

When you press the **Start Design** button in the Design Parameters dialog box, Vector NTI begins the Design process. An interim "Designing..." dialog box appears, showing you the progress of the design and allowing you to break and accept at certain points, or cancel the design entirely.

The design process consists of several stages:

1. Vector NTI collects all the information it needs to perform the design. The message "Collecting necessary data..." is displayed on the screen.
2. Vector NTI creates restriction maps for all molecules, which may be involved in the design. If many maps need to be created (for example, if you chose a large subbase of molecules as potential donors) and if many restriction endonucleases are involved, Vector NTI may take a relatively long time to create all the maps.

3. Vector NTI begins to perform cloning steps. In each step, one donor fragment is cloned into the recipient.
4. When each cloning step is complete, Vector NTI constructs the best recombinant it obtained for that step, models cloning analysis after transformation, searches for important restriction sites in the recombinant, etc.
5. If a cloning step produces the goal molecule, that is, if no donors remain to be cloned, Vector NTI finishes the Design task and enters the goal molecule into the database.
6. If donor fragments remain, the system moves on to the next cloning step.
7. When the Design process is complete, all intermediate recombinants (if any) are stored in a new subbase in the database, called *name_ \$Intermediate*, where *name* is the goal molecule's name. A Molecule Display window opens only for the final molecule.

If you start a Design task for a goal molecule with a name that causes a conflict with an existing set of intermediate recombinants, Vector NTI informs you of the conflict and ask permission to delete the existing intermediates and turn the existing designed molecule into a basic molecule. If you do not want to do this, choose a different name for the new goal molecule.

The recombinants are named as follows: *name_ \$step number_ variant number*, where *name* is the goal molecule's name, *step number* is the number of the cloning step, and *variant number* is the number of the variant of this step, if more than one variant has been generated.

From a special subbase created for the Design result molecules, you can open, examine and/or delete the intermediate recombinants as desired. *If you delete intermediate recombinants, the goal molecule becomes a basic molecule.*

From the moment Vector NTI begins making restriction maps, the **Cancel** button is active. Press it if you want to abort the design at any point. If you cancel the design, the information in the Design Molecule dialog box remains unchanged.

During each cloning step, if at least one acceptable design option has been found, the **Accept Now** button is active. Press it to stop further search and accept the best option already found for the current cloning step. You may wish to turn off the **Immediate Break When There Are Enough Backtracking Options** check box in the Advanced Design Preferences, and control the depth of the heuristic search for each cloning step with the **Accept Now** button.

Returning to the Pre-Design State

When Vector NTI designs molecules, it saves the original goal description (list of recipient and donor fragments) in the database along with all other information about the new molecule. After the molecule has been designed, you can return it to its original state.

This is useful if you want to try several different designs based on the same goal molecule description. Design the molecule one way and inspect the results in a Molecule Display

window. If you are not satisfied with the results, change the design parameters or design preferences, and try again until you are satisfied,

Select **Molecule > Operations > Advanced DNA/RNA > Design**. An intercept dialog box opens reminding you that you will be overwriting the new molecule with the original fragments. Click **Yes** to continue with the new Design. The Lists/Design Molecule dialog box opens again loaded with the original Design component fragments you selected.

If you cannot achieve a satisfactory design by changing the design parameters and preferences, try changing the description of the goal molecule. Change the molecule names each time so you will have a record of your attempts.

If you still cannot achieve a satisfactory design, try changing the internal design parameters to influence Vector NTI's heuristic searches for design options.

The Design Description Folder

The Molecule Display window for the final new molecule automatically opens upon the conclusion of Design. At a later time, you can open a Molecule Display window for newly designed intermediate molecules stored in the database. In either case, the Text Pane of the display window contains a Design Description folder with subfolders for each cloning step used to create the molecule. In each cloning step, one donor fragment is cloned into the recipient.

Cloning Step Subfolders

Each cloning step subfolder of the Design Description folder contains the following information:

- **Result Molecule:** Name of the recombinant obtained in this cloning step. If this is the final cloning step, the name of the recombinant is the same as the name of the goal molecule. If this is an intermediate recombinant, the name is formed according to the rules given previously in this chapter.
- **Recipient**
 - The name of the molecule used as the recipient
 - The restriction sites used as the left and right termini
 - If and how the termini were modified
 - If partial digestion was used in this cloning step, the number of sites inside the required fragment and the total number of sites on the molecule
- **Donor**
 - The molecule used as the donor
 - Whether the fragment was inserted in complementary orientation,
 - The restriction sites used as the left and right termini of the donor

- If and how the termini were modified
- If partial digestion was used in this cloning step, the number of sites inside the required fragment and the total number on the molecule
- The actual flank regions on each end of the donor fragment, that is, the distance in nucleotides between the first or last nucleotide in the fragment and the appropriate boundary of the nearest functional signal
- If the donor fragment was obtained through PCR amplification, the PCR primers used are displayed in the 5'-3' direction. If a restriction site was attached to the 5' end of the primer, that site is also shown here.
- **Ligation Junction types:** cohesive or blunt.
- **Lost restriction sites on the recipient** indicates sites lost during cloning
- **Donor fragment orientation** indicates if the ligation conditions prevent parasitic donor fragment orientations.
- **Preselection** The recommended preselection method
 - If one or both recipient sites are lost after ligation, they will be recommended for effective preselection.
 - If sites are not lost, the system will recommend dephosphorylation (if that was permitted in the Design Parameters dialog box).

The ability to perform preselection influences the choice of methods of clone analysis after transformation.

- **Transformation system:** The recommended transformation system for clone analysis. This information is useful if the user allowed more than one transformation system to be used. Vector NTI considers the size of recombinants, their replicon types, and extra-chromosome replication capabilities.
- **Recommended method(s) for clone analysis:** Recommended methods for clone analysis after transformation. Vector NTI proposes the most reasonable set of four possible methods: Screening, Colony Hybridization, Restriction Analysis, and PCR amplification of the cloned fragment. These recommended methods are based on a number of conditions: ligation type, full or partial digestion, preselection, etc.
- **Recommendations for restriction analysis:** The recommended restriction sites give distinguishable gel patterns for the result molecule (recipient with insertion) and the recipient without insertion.

The following information is given even if these methods were not recommended for clone analysis.

- **Recommended oligonucleotide for colony hybridization:** The oligonucleotide will successfully hybridize with the inserted fragment.

Chapter 27 AlignX

Introduction

The simultaneous alignment of many nucleotide or amino acid sequences is an essential tool in molecular biology. Multiple alignments are used to find diagnostic patterns, to characterize protein families, and to detect or demonstrate a similarity between new sequences and existing families of sequences. They are also useful for predicting secondary and tertiary structures of new sequences, for suggesting oligonucleotide primers for PCR and serving as an essential prelude to molecular evolutionary analysis.

The multiple sequence alignment application, AlignX, of the Vector NTI Suite is a comprehensive program designed to conduct and manage multiple sequence alignment projects. Align X uses a modified Clustal W algorithm (see page 424) and incorporates the following features:

- Profile alignment
- Phylogenetic tree construction, displayed in graphical representation
- Use of residue substitution matrices
- Secondary structure consideration
- Multicolored alignment presentation
- Automatic consensus calculation
- Full alignment editing capabilities
- Dot Matrix comparison of any two sequences

Launching Align X

To launch AlignX, double-click its icon in the program group or folder in which you installed the Vector NTI Suite. From Vector NTI Database Explorer, select **Align > AlignX-Open New Alignment Window** or select a group of molecules then choose **Align > AlignX-Align Selected Molecules**. You can also drag the selected molecules from the Database Explorer into the AlignX workspace. From other Vector NTI applications, select the molecules to be included in the alignment and choose **Align > AlignX – Align Selected Molecules**.

Features of the AlignX Workspace

When AlignX is first opened, the display window is empty. As molecules are added to an AlignX project, they are listed in the Text Pane. After aligning a group of molecules selected in the Text Pane, alignment results then appear in the Graphics Pane and the Alignment Pane.

Because it will be much easier for you to understand the AlignX features in a complete display window, open a demo project into the workspace. Select **Project > Open**. Find the Vector NTI Suite folder. (It is probably located in Program Files\Vector NTIDemo Projects.) Open the Protein.apr file, a sample alignment of 25 molecules.

The AlignX interface (Fig. 27.1) consists of a menu bar, three toolbars, an AlignX Display window, with shortcut menus specific for each pane. A Dot Matrix window can be opened from the AlignX display window when the Dot Matrix tool is launched.

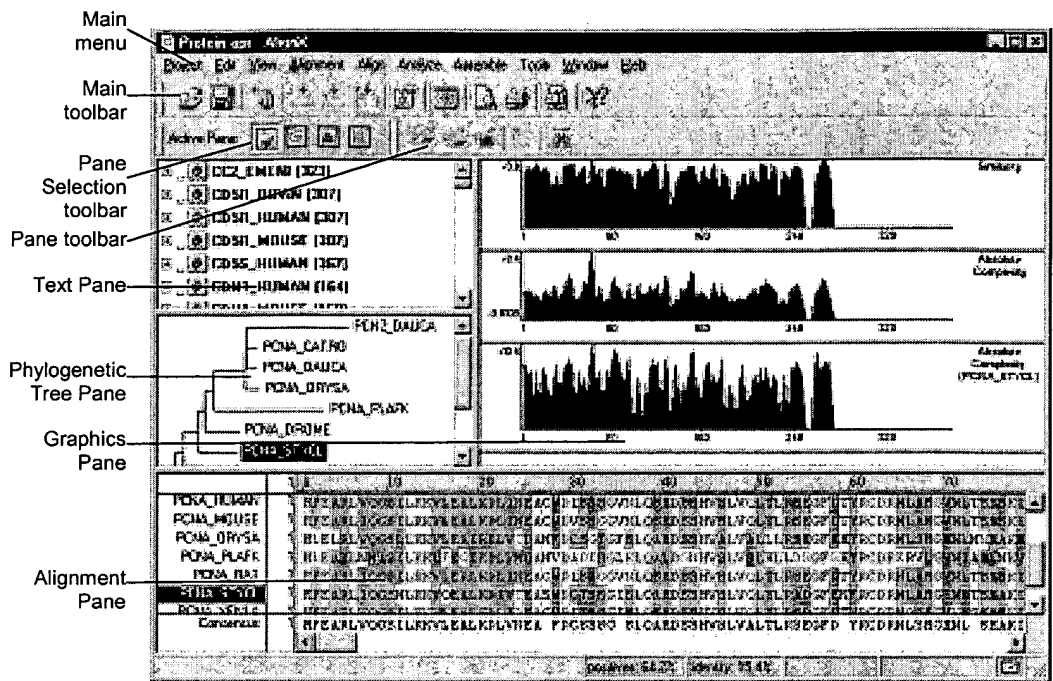






Fig. 27. 1 AlignX interface

AlignX has three toolbars: the Main Toolbar, the Pane Selection Toolbar and a Pane Toolbar. The Main Toolbar contains tools for major program functions, such as opening and saving projects, adding molecules to the project, exporting molecules' data in various formats, and creating alignments.

All Vector NTI Suite toolbar buttons are summarized in Chapter 4 of this manual. As you are introduced to various operations in this chapter, however, the toolbar buttons you will use are displayed appropriately. Many toolbar commands can also be launched from the menu bar or from a shortcut menu.

The AlignX Display window is divided into four panes: a Text Pane, a Phylogenetic Tree Pane, a Graphics Pane, and an Alignment Pane separated by split bars. To apply a command from the main menu in a given pane (Print, Print Preview, Camera), that pane must be

active. The active pane can be toggled with the Switch Panes (, , , ) buttons in the Pane Selection Toolbar or by clicking anywhere in the pane you want to activate.

Split bars allow re-sizing of the four panes in the AlignX Display window. Scroll bars appear when the contents of a pane exceed the viewing area, facilitating easier viewing of pane contents.

Text Pane

The Text Pane contains a thorough description of each molecule included in the alignment project. The information is organized in folders, named by molecule. Each top level (molecule) folder contains the following subfolders:

<i>Folder</i>	<i>Contents</i>
General description	Molecule type, form (for DNA only), length, etc.
Proprietary fields	User defined fields (for molecules which came from Vector NTI)
Standard fields	GenBank/SWISS-PROT-like fields: keywords, division, original accession numbers, etc
Comment	Arbitrary text of any length associated with the molecule
References	Bibliographic references (in GenBank/SWISS-PROT format)
Feature table	List of molecule features

Table 27. 1 AlignX Text Pane folders


Folders and sub-folders can be opened with a double-click or by clicking on the + to the left of the folder name.

For more information regarding the following Text Pane manipulations, refer to Chapter 3.

- Expanding and collapsing folders
- Shortcut menus
- Copying pane contents to the clipboard

Finding Signals on Graphical and Alignment Panes


Signals listed in the Feature map subfolder in the Text Pane can be found on molecule graphics and/or sequences in the graphics and alignments panes (only for those molecules that are part of the current assembly). To visualize the positions of a signal in these panes,

select the feature in the Text Pane folder, then press the **Find** button () on the Text Pane toolbar or select **Edit > Find** on the menu bar. *The Find command is disabled if selected line is not a signal with non-zero boundaries.*

Phylogenetic Tree Pane

Phylogenetic analysis is the means of studying presumed evolutionary relationships. An inferred evolutionary history is displayed in a treelike diagram suggesting the inherited relationships between the molecules.


If there are more than 3 sequences in an alignment, a phylogenetic tree is shown in the

Phylogenetic Tree Pane. To activate this pane, click the **Phylogenetic Tree** button () on the Pane Selection Toolbar. Scrollbars in the Phylogenetic Tree Pane enable you to view the entire tree.

A phylogenetic tree in the Vector NTI Suite is built using the Neighbor Joining method (NJ) of Saitou and Nei. The NJ method works on a matrix of distances between all pairs of sequence to be analyzed. These distances are related to the degree of divergence between the sequences. The phylogenetic tree is calculated after the sequences are aligned.

Exporting a Phylogenetic Tree


To export the phylogenetic tree into a .ph file for use with other applications, press the

() button on the Phylogenetic Tree Pane Toolbar or choose **View > Export Phylogenetic Tree** on the menu bar or select Export Phylogenetic Tree from the shortcut menu. Select or enter the file name to export to in the File Save dialog and press **Save**.

Interconnection with Other Panes

Any molecule selected in the Phylogenetic Tree Pane is selected concurrently in the Alignment Pane.

Graphics Pane

To activate the Graphics Pane, click on the **Graphics Pane** button () on the Pane Selection Toolbar. The graphical representation of an alignment contains three graphs in the Graphics Pane. Graphs are divided by horizontal split bars that can be moved to change the height of the display area.


Descriptions of the Graphs

- **Alignment Quality Profile** (upper graph): Specific values (in a 0-1 range) are assigned to each residue at a given alignment position in each aligned sequence, depending on whether the residue is identical, similar or weakly similar to the corresponding residue of the consensus sequence. The values (1 (identical), 0.5 (similar), and 0.2 (weakly similar) for each residue at a given position are totaled; the sum is divided by the number of the sequences in the alignment, “normalizing” the resulting value.
- **Absolute Complexity Profile of the Multiple Alignment:** (center graph): This graph is calculated as a sum of all pairwise residue substitution scores at a given alignment position divided by the number of pairs in the alignment. The scores are taken from the residue substitution matrix used for alignment calculation.

- **Absolute Complexity Profile of a Pairwise Alignment** [for the selected molecule relative to the consensus sequence] (lower graph): This graph is calculated in a manner identical to the one in the second graph. Selection of a molecule in any pane results in the calculation of this graph. This graph is the only one that changes according to which molecule is selected.

All graphs display the values averaged in a window of a specific length (defined by "window" parameter) that slides along the alignment.

You can add additional analyses to the graphics pane by activating the Graphics Pane and choosing **View > List of Analyses** from the drop down menu or press the **Analysis List**

button () to open the Analysis List setup dialog box (Fig. 27.2):

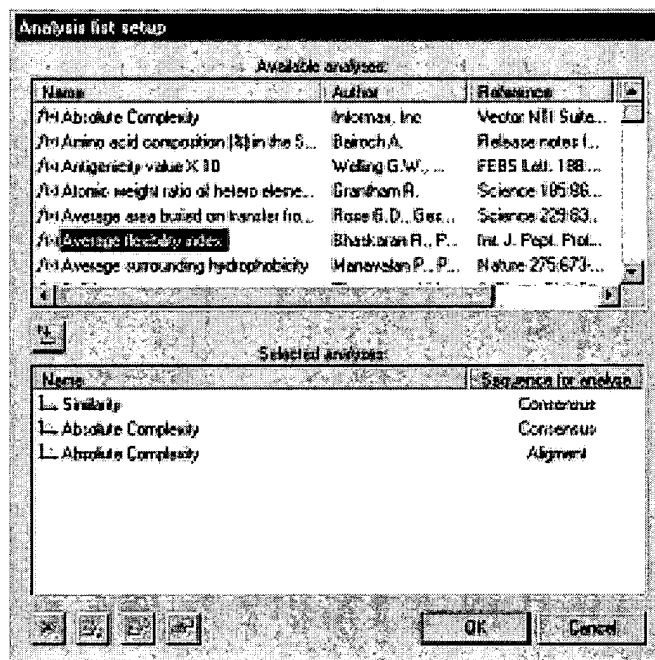




Fig. 27. 2 Analysis List Setup dialog box

The dialog box lists all of the analyses available for DNA or protein, depending on the type of aligned molecules. Currently displayed analyses are listed in the Selected Analyses box. To add an analysis, select it in the Available Analyses list box and click the **Add Analysis**

button () to move it to the Selected Analyses list. Alternately, you can move an analysis to the lower list by double clicking on it.

The plot display order is determined by the order the analyses are listed in the Analysis List setup box. To modify the order, select an analysis and move it up by clicking the **Move Up**

button ). Click **OK** to close the Analysis list setup dialog box and generate the new analyses.

Adding Custom Protein Analyses

Specific values are used in the calculation of the various protein analyses. These values differ depending on the particular analysis and are stored in small text files having a .pa2 extension. All .pa2 analysis files are stored in the Analyses subdirectory in the Vector NTI Suite directory. The name of the analysis corresponds with the name of the .pa2 file.

For example, the Bulkiness analysis is characterized in the Bulkiness.pa2 file as follows:

```
[general]
author=Zimmerman J.M., Eliezer N., Simha R.
comment=Last modified 15/Mar/1996 by ELG
reference=J. Theor. Biol. 21:170-201(1968).
unique=s30
[data]
values=11.5,14.28,12.82,11.68,13.46,14.45,13.57,3.4,13.69,21.4,21.4,15.71,16.25,19.8,
17.43,9.47,15.77,21.67,18.03,21.57
```

The [general] section describes the attributes of the analysis and the [data] section contains the list of values used for calculating the analysis. Values are listed according to the alphabetical order of the acid coefficients.

You can create your own custom protein analysis by modifying the values in one of the existing .pa2 files using a plain text editor, such as Notepad. Once modified, the new .pa2 file should be saved in the Analyses subdirectory under a new name that corresponds to the new analysis type. If AlignX is open at the time the new .pa2 file is created, it must be closed and reopened before the new analysis is recognized.

Plot Setup

To modify a particular graph in the Graphics Pane, click on the graph you want to change and choose **View > Plot Setup** or right click on the graph you want to change and choose Plot Setup from the shortcut menu.

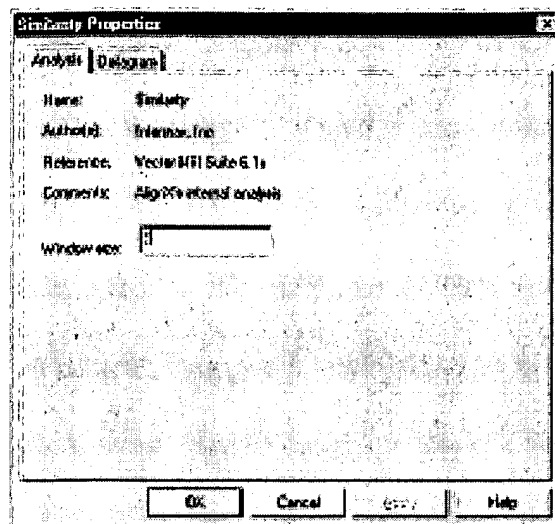


Fig. 27.3 Analysis tab of Plot Setup dialog box

On the **Analysis** tab of the Plot Setup dialog box (Fig. 27.3), you can set the Window size, that is the number of sequential residues used to calculate the average for each graph point. The larger the number entered (Window size), the smoother the graph curve.

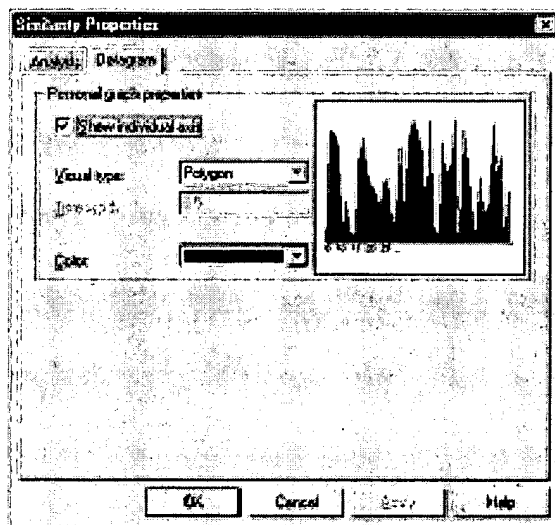


Fig. 27.4 Datagram tab of the Plot Setup dialog box

On the **Datagram** tab of the Plot Setup dialog box (Fig. 27.4), you can modify the plot color. Both the size of the window and plot color you select are specific for the selected

graph in the current project. You can also change the graph style and choose to show or hide the horizontal axis for that graph here. These settings are stored within the project file and applied to graph next time you load the project.

Zooming Graphs In and Out

Enlarging or reducing regions of alignment graphs is possible using the Zoom buttons. For descriptions of their use, refer to Chapter 3.

Managing the Scales of the Graphs

Each graph has its own vertical and horizontal scales. Vertical scales are similar for each graph and show the maximum and minimum values for the graph. On the horizontal scale, either numerical positions in the sequence or residues can be shown, depending on the degree of zooming in the graphics pane. If you would like to see the residue designations, zoom in on the graphics pane. The vertical and horizontal scales can be further managed as follows:

- As mentioned above, the horizontal axis can be shown or hidden for a particular graph by checking or unchecking the Show individual axis box on the **Datagram** tab of the Plot Setup dialog box.
- The **Show Vertical Axis** command is used to show or hide the vertical axis for all the graphs. This command can be accessed from a button on the tool bar or the shortcut menu launched by right clicking on the Graphics pane.


Graph legends can be removed from the pane by unselecting the **Show Legends** button from the Pane Toolbar or by unchecking the **Show Legend** command from the shortcut menu or the View menu. The **Place Legends Below Plots** command is used to toggle the graph legends display from the right side of the graphs to below the graphs. The **Place Legends Below Plots** command can be accessed either from the shortcut menu or from a button on the toolbar.

If no items are checked, the graphs are shown without annotation.

Interconnection with Alignment Pane

Selections within the Graphics Pane are concurrently selected in the Alignment Pane. In general, regions can be selected using the click + drag technique. Detailed selection techniques are discussed in Chapter 3.

Alignment Pane

To activate the Alignment Pane, click on the **Alignment Pane** button () on the Pane Selection Toolbar.

The Alignment Pane displays aligned sequences and the resulting consensus sequence. *Consensus residues are those that appear most commonly at a particular site.* The Alignment Pane has its own vertical and horizontal scrollbars. The last row in the pane consists of the alignment consensus.

Vertical scrolling affects both the aligned sequences and their names, but the consensus sequence is not included in the scroll region: it remains visible as the bottom sequence. The sequence names remain stationary at the left of the Alignment Pane no matter how the sequences are repositioned horizontally. If the list of molecules listed in the Alignment Pane is longer than the size of the pane, a vertical scroll bar appears as well. Vertical scrolling affects both sequences and their names but the consensus sequence remains visible at the bottom of the pane.

AlignX shows similarity and identity levels for the generated alignment in the Alignment Pane. The Status bar displays the percent of similar and identical residues for entire alignment or for a selected region on the alignment. When the mouse cursor is pointed to any particular column within alignment, a popup label shows the percent of similar and identical residues within an alignment column. *The popup label option is disabled if only two sequences are aligned.*


Interconnection with Other Panes

When a molecule is selected in the Alignment Pane, it is selected in the Phylogenetic Tree Pane as well. *No phylogenetic tree is calculated when there are three or less sequences in the alignment.*

A selected region within the Alignment Pane is also selected concurrently in the Graphics Pane.

Alignment Display Setup

To change a default set of alignment display parameters, press the **Alignment Display**

Setup button () on the Alignment Pane toolbar or choose **View > Display Setup** from the menu bar or **Display Setup** from the shortcut menu. The Alignment Display Setup dialog box opens with three property tabs: Consensus Calculation, Similarity Tables, and Color Setup.

Consensus Calculation

A consensus sequence is a theoretical representative nucleotide sequence in which each nucleotide represents either the residue seen most frequently at that same site in aligned sequences, or is selected by other criteria. The Consensus Calculation tab specifies how the consensus sequence, displayed as the bottom sequence in the Alignment Pane, is calculated in Align X.

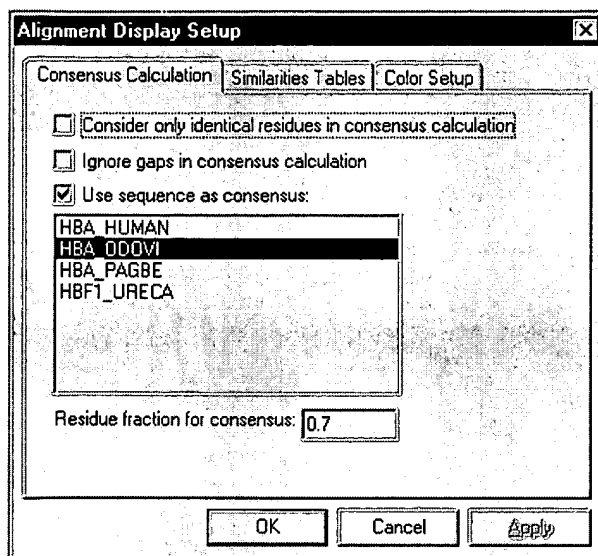


Fig. 27. 5 Consensus Calculation tab of Alignment Display Setup dialog box

On the Consensus Calculation tab (Fig. 27.5), you can select among the following parameters:

- Consider only identical residues...
- Ignore gaps in the consensus calculation
- Specify a sequence to use as the consensus sequence. In this case, select one of the sequences listed (those used in the alignment).
- Residue fraction

If the **Use sequence as consensus** checkbox is checked, the Consensus will not be calculated but the sequence selected in the listbox below the checkbox will be used as the consensus.

Similarity Tables

The Similarity Table (Fig. 27.6) allows you to review or to define residue similarity information and values necessary for calculation of the alignment quality profile. Each residue pair can be defined as being strongly or weakly similar to each other. *The table is editable only for amino acid residues.*

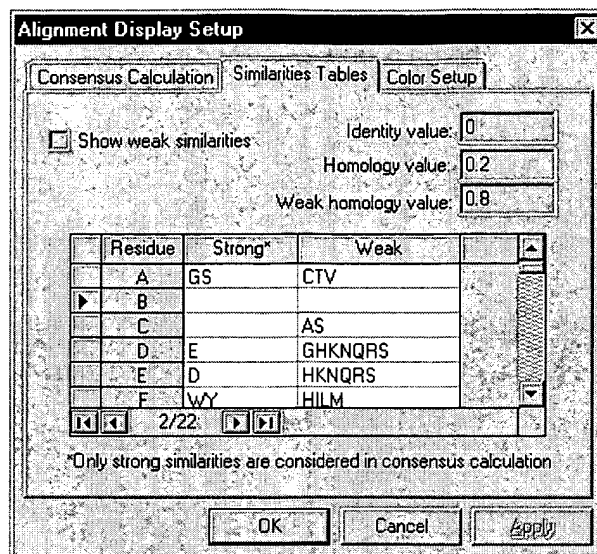


Fig. 27. 6 Similarity Tables tab of Alignment Display Setup dialog box

Alignment Color Scheme Setup

Residues in an alignment are colored according to the following scheme:

Color	Description
black on window default color	non-similar residues
blue on cyan	consensus residue derived from a block of similar residues at a given position
black on green	consensus residue derived from the occurrence of greater than 50% of a single residue at a given position
red on yellow	consensus residue derived from a completely conserved residue at a given position
green on window default color	residue weakly similar to consensus residue at given position

Table 27. 2 Alignment color scheme

To modify alignment display colors, in the Alignment Display Setup dialog box, select the Color Setup tab (Fig. 27.7).

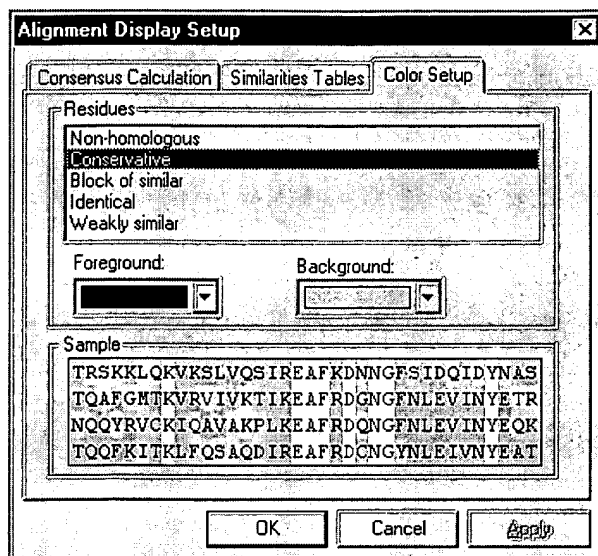


Fig. 27. 7 Color Setup tab of Alignment Display Setup dialog box

Change the colors for the specific project using the drop-down menus, observing the modifications in the preview box. Click **OK** to save color changes in the alignment project. They will be applied to the alignment next time you open the project.

After any changes are saved, consensus and colors for residues in the Alignment Pane are recalculated as well as values for all the three graphs in the Graphics Pane, and both panes are redrawn.

AlignX Projects


Creating a New Project

When AlignX is launched, a new, empty alignment workspace is created. To create a new alignment project while in the AlignX display window, select **Project > New** on the menu bar. *If you have any unsaved changes in an existing, and currently open project, AlignX suggests changes should be saved.*

In the dialog box that opens, select **Yes** to save changes, **No** to disregard changes or **Cancel** to discard changes and continue work on the opened project. In the first two cases, the current project will be closed and the workspace emptied.

Opening existing project

To open an existing project, choose **Project > Open** on the menu bar or press the **Open**


Project button () on the Main Toolbar. In the Open dialog box, select the desired file and press **Open**. *By default, alignment projects have an "*.apr" file extension.* If the file is

an alignment project, information about molecules, properties and alignments (if any) appears in appropriate panes.

Another way to open an existing project is to drag and drop the project file on the AlignX workspace.

Adding Molecules to the Project

Molecules for a new (or existing) project can be added to AlignX in these ways:

- In the Vector NTI Database Explorer, in either a DNA/RNA subbase or a protein subbase, select a list of molecules, and drag them into the Text Pane of the empty AlignX workspace. Molecules can be dropped in the AlignX workspace to be added to the project if they are of an appropriate file format and molecule type.
- Select **Project > Add files** on the AlignX menu bar or click the **Add Files** button () on the Main Toolbar. Choose the appropriate file of molecules from the Add files to project dialog box, and click **Open**.

AlignX reads various file formats: GenBank and EMBL for DNA, GenPept and SWISS-PROT for proteins, FASTA and Vector NTI archives for both DNA and proteins. The first added molecule determines the molecule type. If you try to add molecules of a different type later, a warning message appears and the inappropriate molecule is not added. If a molecule's type correlates with the current project, the molecule(s) are added to the project and appear in the Text Pane.

If the program fails to define a file's format, the file can be imported as a raw sequence. If there are no molecules in the project, the molecule's type must be selected before it is possible to import the file (Fig. 27.8). If the project is not empty, the molecule type is already defined and the raw sequence can be imported only if its file type matches the current project.

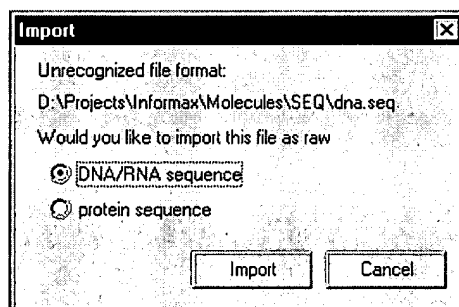


Fig. 27. 8 Importing a raw sequence

Imported raw sequences appear in the Text Pane with the names such as NONAME, and NONAME#2. Select **Rename Molecule** from the shortcut menu to rename the molecule.

Even after molecules are brought in the AlignX workspace, three of the panes are empty until the alignment is created.

Alignment Import in MSF Format

In addition to its own projects, AlignX can open alignments of the MSF format. To open an MSF project, choose **Project > Import MSF Format** from the menu bar. In the File Open dialog box, select the MSF project to be opened. *By default, MSF projects have an .msf extension, but are not limited to it.* AlignX tries to recognize the MSF format itself, not simply by its file extension. You can also open an MSF project by dragging the file and dropping it within the AlignX window. An MSF project imported into a new AlignX project is initially named **untitled**. Select **Rename Molecule** from the shortcut menu to rename the molecule.


The information contained within an MSF project is molecule type, name, length and sequence. For this reason, Text Pane folders of MSF-format molecules contain no information about creation and modification date, author, original access number, references, and feature tables. Work is done with an MSF project just like any other AlignX project.

Initiating an Alignment


There are two ways to initiate alignments. In the Text Pane, select the sequences to be included in the alignment, then proceed with either a multiple alignment, or a profile alignment.

Multiple Alignment

In a multiple sequence alignment in AlignX, all sequences included in the alignment are “equally important.” AlignX uses a modified Clustal W algorithm in which pairwise alignments are performed between sequences and the alignment tree proceeds using the particular scoring matrix designated by the user in Alignment Setup (see page 431), whereas in the traditional Clustal W, the algorithm chooses the scoring matrix or series of matrices as the alignment progresses. (See page 715 for alignment algorithm references.)

To initiate a multiple alignment, press the **Align** button () on the Main Toolbar or select **Alignment > Align Selected Sequences** from the menu bar. *This function is enabled only if more than one molecule is selected in the Text Pane.*

Profile Alignment

A profile alignment in AlignX is based on aligning all selected sequences against a reference sequence. This is based on the ClustalW algorithm. To initiate a profile alignment, press the **Align Profile** button () on the Main Toolbar or choose **Alignment > Align Selected Sequences Using Profile** on the menu bar. In the Select Profile dialog box (Fig. 27.9) that opens, select the molecule to be used as the first profile.

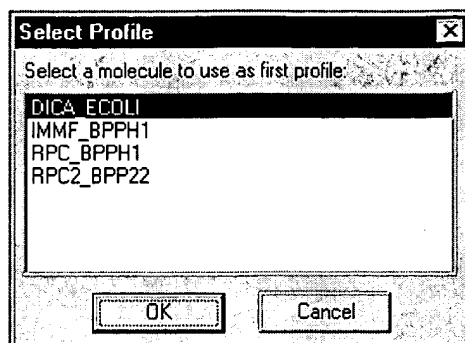




Fig. 27. 9 Select Profile dialog box

This function is available only if more than 1 molecule is selected in the text view.


Adding a Molecule to an Alignment

To add a molecule to the alignment, select the molecule in the Text Pane or drag it in from Database Explorer. Press the **Add to Alignment** button () or choose **Alignment > Add Selected To Alignment**. A profile alignment using an existing alignment as the first profile will be performed. This function is available when an alignment already exists and at least one molecule that does not belong to alignment is selected in the Text Pane.


Removing a Molecule from an Alignment

To remove a selected molecule from the alignment (but retain it in the project), press the **Remove Molecule from Alignment** button () on the respective pane's toolbar or choose **View > Remove <molecule name> From Alignment** or select the same command from the shortcut menu. If you confirm removal of the molecule, the specified molecule is removed and the phylogenetic tree, consensus and graphs are all recalculated the panes refreshed. A molecule can be selected for removal either in the Text Pane, the Phylogenetic Tree Pane or the Alignment Pane.

Removing a Molecule from the Project

To delete a selected molecule from the project, press the **Delete Molecule from the Project** button () from the Text Pane toolbar, choose **View > Delete From Project** on the menu bar or **Delete From Project** from the shortcut menu. If you confirm this operation, the specified molecule will be deleted from the project. If the molecule is removed from the alignment, the phylogenetic tree, consensus and graphs are recalculated and refreshed.

Setting Alignment Parameters

The first molecule added to an alignment defines the molecule type of the alignment (DNA or protein). When initiated, a default set of alignment options is created for the defined molecule type. To change these options, press the **Alignment Setup** button () on the

Main Toolbar or choose **Alignment > Alignment Setup**. The Alignment Parameters dialog box opens with five property tabs: Pairwise Alignment, Multiple Alignment, Protein Profile Alignment, Tree Construction Options and Score Matrix (Fig. 27.10).

Pairwise Alignment

The method for calculating pairwise distances is defined on the Pairwise Alignment tab.

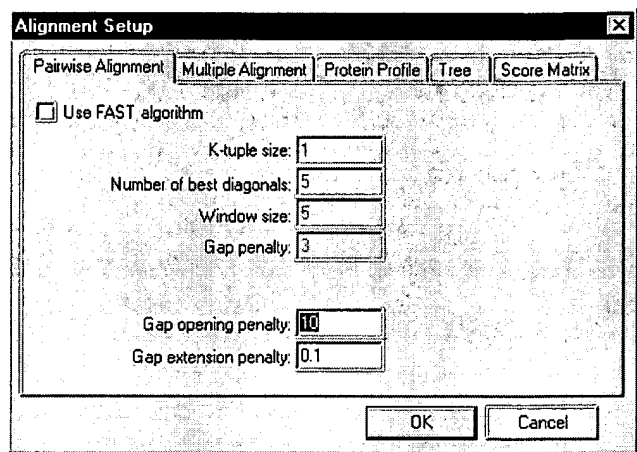


Fig. 27. 10 Alignment Setup dialog box, with Pairwise Alignment tab displayed

Pairwise alignment parameters control the speed/sensitivity of the initial alignments:

- Fast (approximate) method
- Slow (more accurate) method uses two gap penalties (for opening or extending gaps) and a full amino acid weight matrix.

By default, the slower method is used. There are two groups of parameters enabled on this pairwise tab depending upon which method is chosen.

Note: Default settings are in parentheses: first number = DNA; second number = protein

Pairwise Alignment Parameters	
Slow Options	
Gap opening penalty (15/10)	The penalty for the first residue in a gap
Gap extension penalty (6.66/0.1)	The penalty for additional residues in a gap

<i>Pairwise Alignment Parameters</i>	
Fast Options	
Number of K-tuple matches (2/1)	Change the K-tuple value to limit the word-length the search should use. A word-length of 2 is sensitive enough for most protein database searches. The general rule is that the larger the word length, the less sensitive, but faster the search will be.
Number of best diagonals (4/5)	Number of the k-tuple matches on each diagonal used in the alignment
Window size (4/5)	The number of diagonals around each of the best diagonals used
Gap penalty (5/3)	Penalty for the existence of a gap

Table 27. 3 Pairwise Alignment parameters

Multiple Alignment

These parameters control the final multiple alignment (Fig. 27.11).

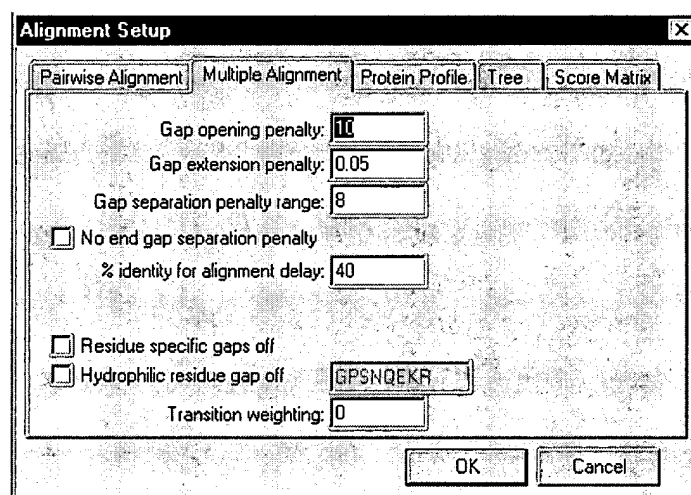


Fig. 27. 11 Multiple Alignment tab of Alignment Setup dialog box

Note: Default settings are in parentheses: first number = DNA; second number = protein

Multiple Alignment Parameters	
Gap opening penalty (15/10)	The penalty for the first residue in a gap
Gap extension penalty (6.66/0.05)	The penalty for additional residues in a gap
Gap separation penalty range (8)	Tries to decrease the distances between gaps
Use end gap separation penalty	Does not penalize for gaps introduced at the end of a sequence
% identity for alignment delay (40)	Delays the alignment of the most divergent sequences; that is, sequences that are less identical than the percentage shown will be introduced into the alignment later.
Use residue-specific gaps	Amino acid-specific penalties that increase or reduce penalties at each position in the aligned sequence
Use hydrophilic residue gap	Increases the chance of a gap within a region of hydrophilic residues (if yes, specify in the following text box)
Transition weighing	(For DNA only) Gives translations (A<->G or C<->T) a specific weight. (0) means the transition is treated as a mismatch while 1 gives the transition a match score (0)

Table 27. 4 Multiple Alignment parameters

Protein Profile Alignment

This dialog box allows you to raise penalties for opening gaps in secondary structure (protein) regions (Fig. 27.12).

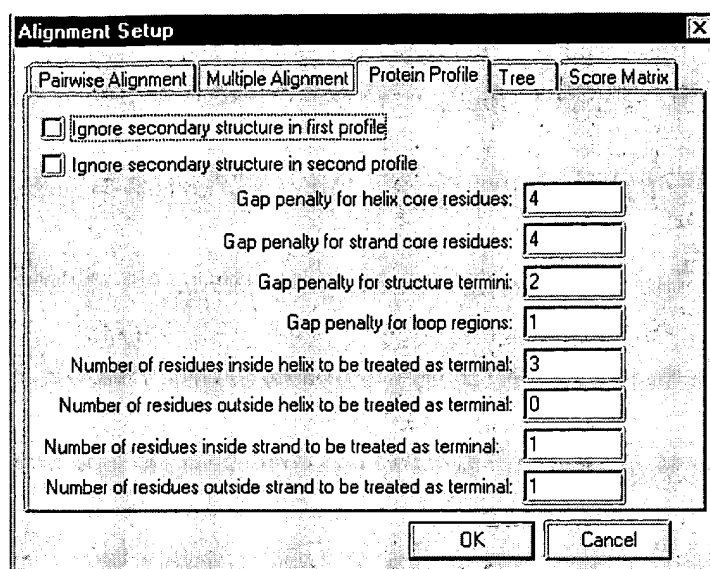


Fig. 27. 12 Protein Profile tab of Alignment Setup dialog box

Note: Default settings are in parentheses.

Profile Alignment Parameters	
Use secondary structure in first/second profile (yes or no) [If no in either profile, all other parameters are disabled]	Determines if the secondary structure information is to be used in the indicated profile
Gap penalty for helix core residue (4)	For helix core residues breaking
Gap penalty for strand core residue (4)	
Gap penalty for structure termini (2)	For introducing gaps at the end of a secondary structure
Gap penalty for loop regions (1)	Raises the penalty for introducing gaps in a loop (By default this penalty is not raised.)

Profile Alignment Parameters	
Number of residues inside/outside a helix/strand to be treated as terminal Inside helix (3) Outside helix (0) Inside strand (1) Outside strand (1)	Specifies the range of residues inside or outside a helix or strand beyond the structure

Table 27. 5 Profile Alignment parameters

Phylogenetic Tree Construction Options

A phylogenetic tree is calculated from the alignment using the Neighbor Joining (NJ) method. On the Tree tab (fig. 27.13), two parameters may be changed for tree construction (neither of these are set initially):

Tree Construction Parameters	
Use Kimura's correction	Corrects for the fact that observed distances underestimate the actual evolutionary distance. For small divergence, this option makes no difference. For greater divergence (> 10%), substitutions can occur multiple times at different sites. (Kimura, 1983)
Ignore positions with gaps	When checked, any alignment positions where the sequences have gaps will be ignored.

Table 27. 6 Tree Construction parameters

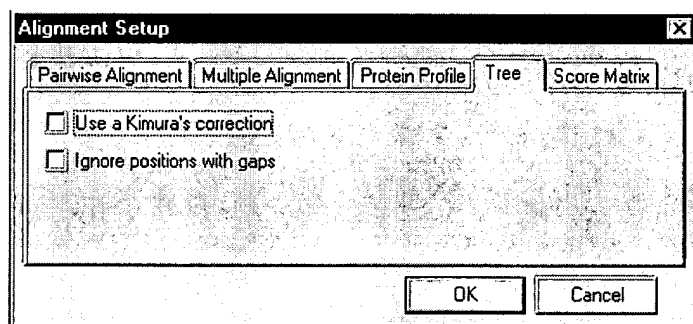


Fig. 27. 13 Tree tab of Alignment Setup dialog box

Score Matrix

The Score Matrix tab (Figure 27.14) displays the matrix used to generate the current multiple alignment. From this tab, you can also open other matrices for review or for application to the alignment.

When a project is created, a residue substitution matrix from the corresponding template (DNA or protein) is used. If either template file is not found, the default matrix is used (swgapdnamt for DNA and blosum62mt2 for protein). To review other matrices for either DNA or proteins, click the **Select Matrix** button. From the Matrices file, select any other matrix to be loaded on the Score Matrix tab.

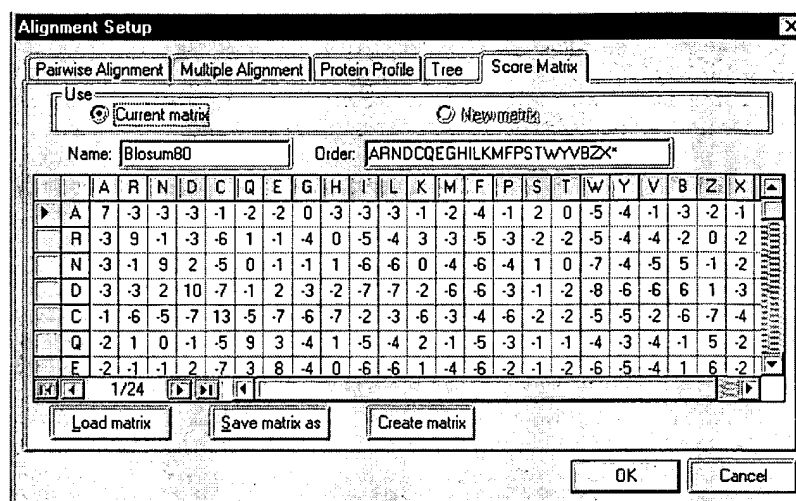



Fig. 27. 14 Score Matrix tab of Alignment Setup dialog box

Editing a matrix takes place in the Matrix Editor, accessible from the program files where you installed the Vector NTI Suite. For further details regarding customizing or editing matrices, refer to Chapter 36.

Editing the Alignment

To edit an alignment, press the **Edit Alignment** button () on the Alignment Pane toolbar, or choose **View > Edit Alignment** from the menu bar or Edit Alignment from the shortcut menu. This opens the Edit Alignment dialog box containing rows of molecule names and sequences (Fig. 27.15).

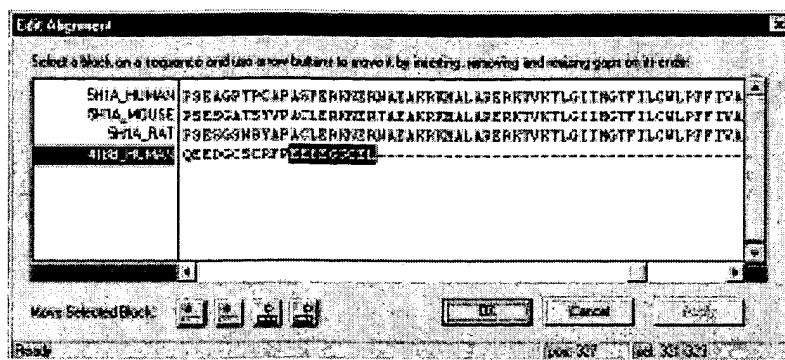


Fig. 27. 15 Edit Alignment dialog box

To edit the alignment, select the desired region in the sequence by LEFT-CLICK + DRAG through the sequence in the appropriate direction with the mouse or double-click with the left mouse button within the sequence, selecting the entire region between two gaps. The current selection, as well as its position in the sequence, is shown in the status bar of the dialog box.

Editing can only occur by shifting selected sequences into adjacent gaps. Therefore, the four buttons allowing movement of the selected block are enabled only when there is a gap to the left or right of the selected region. To shift the block, press one or more of the following buttons:



- to the beginning of the adjacent gap interval in the left direction



- one position left



- one position right



- to the end of the adjacent gap interval in the right direction

Click the **OK** button to save any changes and close the Alignment Edit dialog box. Click the **Apply** button to save any changes and leave the dialog box open. *This button is enabled only when there are differences between the alignment shown in the Alignment Pane and the edited alignment.* The **Cancel** button discards all the changes made since the last use of **Apply**.

Templates

Template files contain all the Alignment Setup, the Plot Setup, or the Alignment Display Setup properties for the AlignX program. When an alignment project is being created, AlignX locates the default template file (default.atp for protein and default.atn for DNA) in

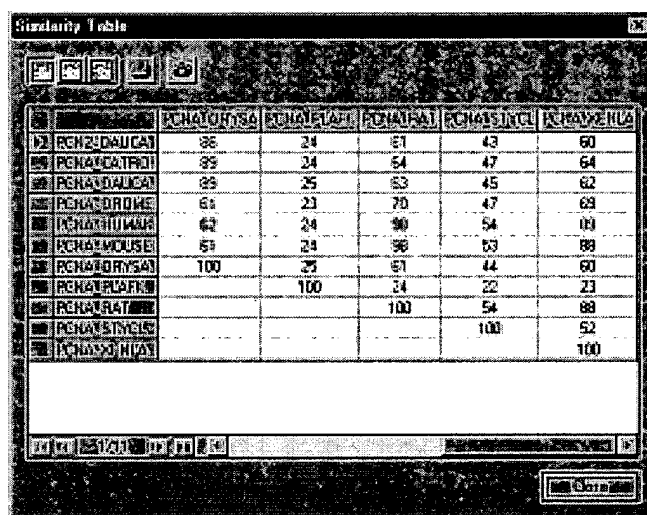
the main Vector NTI directory. If the file exists, the properties from it apply to the project. If the file is not found, default predefined values from AlignX itself are used.

You can save current settings of the alignment project in a template file or apply settings from an existing template to an open project. To save settings as a template, choose **Edit > Setup > Save to File** and select the destination for the settings (or type the name of the new template file). *Default extension for template files is ".atp" for proteins and ".atn" for DNA.* To apply settings from an existing template to the open project, choose **Edit > Settings > Load from File** and select the template file. Settings affecting the plot presentation (Plot Setup) or the alignment presentation (Alignment Display Setup) take effect immediately. The alignment properties (Alignment Setup) take effect only after the alignment is recalculated. To do this, select and realign the same molecules from the Text Pane.

Similarity Table Analysis

The Similarity Table analysis shows similarity and/or divergence values for all possible sequence pairs for the sequences that are included in the alignment.

To open the Similarity Table, choose **Alignment > Show Similarity Table** from the menu. A Similarity Table window opens (Fig. 27.16):




The screenshot shows a window titled "Similarity Table" with a table of similarity values. The table has 6 columns and 6 rows. The first column lists sequence identifiers, and the subsequent columns show similarity percentages for pairs of sequences. The sequences are: PCHADALCAN, PCHADATPRO, PCHADALCAN, PCHADROME, PCHADHUMAN, PCHADMOUSE, PCHADORYSA, PCHADCAFE, PCHADRAT, PCHADTIGER, and PCHADHUMAN. The similarity values are as follows:

	PCHADALCAN	PCHADATPRO	PCHADALCAN	PCHADROME	PCHADHUMAN
PCHADALCAN	86	24	81	43	60
PCHADATPRO	89	24	54	47	64
PCHADALCAN	89	25	53	45	62
PCHADROME	61	23	70	47	69
PCHADHUMAN	62	24	90	54	100
PCHADMOUSE	51	24	98	53	88
PCHADORYSA	100	25	91	44	60
PCHADCAFE		100	24	22	23
PCHADRAT			100	54	88
PCHADTIGER				100	52
PCHADHUMAN					100

Fig. 27. 16 Similarity Table window

There are three different modes of analysis accessible via the buttons on the top of the Similarity Table dialog box. Press the **Top Similarity** button (FS) to show similarity values between the sequence pairs. Press the **Top Divergence** button (FD) to show divergence values for non-identical pairs. Press the **Top-similarity, bottom-divergence**

button () to show pair similarity values at the top of the table and pair divergence values at the bottom of the table. You can also use the **Print** button to print the table or the **Camera** button to copy the table to the Clipboard so that it can be pasted into another application, such as Word or Excel.


Dot Matrix Analysis

The Dot Matrix analysis is primarily a method for comparing two sequences to find all possible matches of residues. This method can also be used to find direct or inverted repeats in protein and DNA sequences. It can predict regions in RNA that are self-complementary and therefore might form a double-stranded region or secondary structure.

In the Dot Matrix method of sequence comparison, one sequence (A) is listed across the top of a page and the other sequence (B) is listed down the left side. Starting with the first positions in A and B, the program slides the window of *n* characters along the sequences performing a comparison of adjacent positions in the windows. If the similarity of residues in each position is above a certain cutoff, a dot is placed in the matrix in the position defined by the starting positions of the window for both sequences. A diagonal line segment indicates that the two sequences match consistently over an extended region.

A larger window size is generally used for DNA sequences than protein sequences since the number of random matches is much greater for DNA.

Launching the Dot Matrix

To perform a Dot Matrix analysis, open a Dot Matrix window by pressing the **Dot Matrix** button () or choosing **Alignment > Show Dot Matrix Plot**. A Dot Matrix window opens in a separate modal popup window.

All molecules present in the alignment plus the consensus are available in both drop-down menus at the top of the empty screen (Fig. 27.17). The molecule selected in the Alignment Pane is shown in the first drop-down menu. The Dot Matrix is calculated when a new selection is made in either drop-down menu (the second should not be empty). The consensus is treated as a common molecule.



Fig. 27. 17 Dot Matrix window drop-down menus

The Dot Matrix window contains a menu bar, Main Toolbar, molecule selection toolbars, status bar, and Dot Matrix Pane. All of the toolbar buttons for the Dot Matrix are displayed and described in Chapter 4. Toolbar buttons are described in this section as needed.

Dot Matrix Pane

The Dot Matrix Pane is represented in the following example (Fig.27.18):

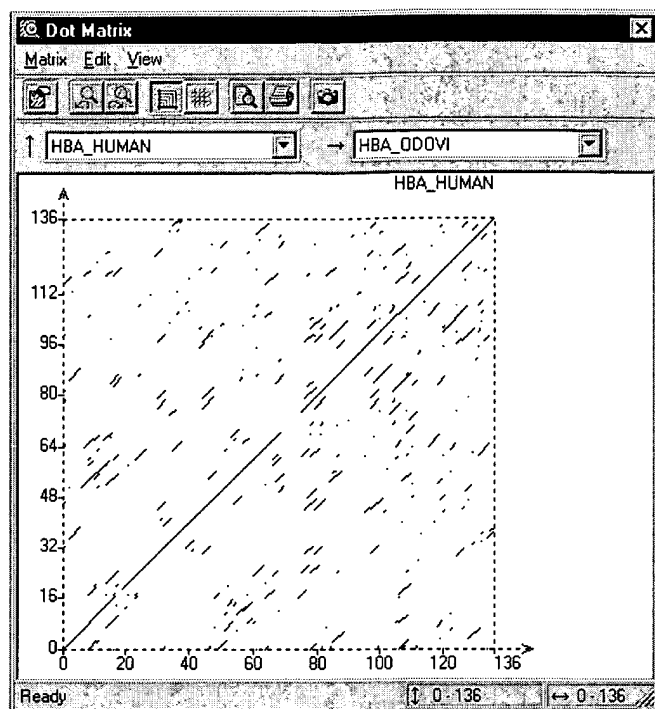




Fig. 27. 18 Dot Matrix Pane



The graphical representation of a Dot Matrix is a dashed rectangle in which any region of similar sequence is revealed by a diagonal row of dots. The molecule selected first corresponds to the horizontal axis, its name being shown in the right top corner of the Dot Matrix rectangle. The second molecule corresponds to the vertical axis. Each axis has its own scale that initially displays the sequence positions. When changing the scales (resizing Dot Matrix Window or zooming Dot Matrix Pane) it is possible to show residues along the axes. Residues are displayed only if they can be displayed on both axes. When the scales allow displaying of residues, a grid can be laid upon the Dot Matrix. To apply (or revoke) a grid, press the  button or choose the Grid on Zoomed Matrix command from the shortcut menu or the View menu.

There are two modes in displaying a Dot Matrix, alternated by pressing the **Isotropic Scaling** button () or selecting **View > Maintain Isotropic Scaling**:


1. Equal scales are used for both axes, meaning the diagonal rows of dots always have a 45° angle relative to the axes.
2. Various scales for axes, that means that Dot Matrix always occupies maximum space in the Dot Matrix Pane.

The scales for X & Y-axes change dynamically when you resize the Dot Matrix Window. Also, the boundaries of regions currently displayed are shown for both molecules.

Resizing the Dot Matrix

When the Dot Matrix is first calculated, it is shown as a representation of the full length of a molecule. Generally it is impossible to analyze molecules of several thousand residues each when displayed in a 500 x 500 (approximate) screen matrix. To alleviate this problem, a region of a Dot Matrix may be enlarged: click and hold the left mouse button within the Dot Matrix rectangle while dragging the mouse, displaying the tracking dotted rectangle. When the mouse button is released, the selected region is enlarged to the entire Dot Matrix rectangle. The previous state is saved in the history list. Click the **Undo Zoom**  and **Redo Zoom**  buttons for modifying the view of the selected region.

Dot Matrix Setup

Open the Dot Matrix setup (Fig. 27.19) by pressing the  button on the main toolbar or by choosing the Matrix Setup command from the Matrix menu.

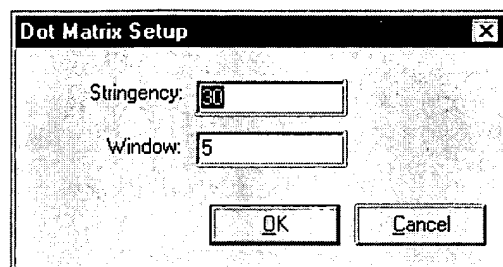


Fig. 27. 19 Dot Matrix Setup dialog box

Two parameters affect the Dot Matrix calculation:

<i>Dot Matrix Setup</i>	
stringency (30% by default)	the minimal number of matches in the window to cause a dot to be set in the matrix
window (5)	the size of the sliding window

Table 27. 7 Dot Matrix setup

Other AlignX Features

The following features can be performed in the AlignX display window. Details specific to AlignX are outlined here. For more information refer to Chapter 3.

- Print and Print Preview
- Copying to the clipboard

Data or graphics from any pane, including the Dot Matrix can be copied to the Clipboard.

- Exporting molecules

*To export a currently open AlignX project into the MSF format, choose **Project > Export MSF Format** and enter the name of the file it is to be saved as.*

- External tools

Alignment Algorithms

For details regarding alignment algorithms, refer to Appendix B.

Chapter 28 BioPlot

Introduction

BioPlot is a sequence analyzer that performs certain types of DNA and protein sequence analyses, and displays the results as linear graphics. For proteins, amino acid scaling allows the user to visualize the distribution of the particular physio-chemical properties along the polypeptide chain, thus providing helpful hints about the functional properties of the protein regions or domains. An amino acid scale is defined by a numerical value assigned to each type of amino acid. The most frequently used scales are the hydrophobicity, hydrophilicity, and the secondary structure conformational parameters scales, but many other scales exist which are based on different chemical and physical properties of the amino acids. This program provides about 60 predefined scales entered from the literature.

Launching BioPlot

To launch BioPlot, select or double-click its icon in the program group or folder in which you installed Vector NTI Suite. To launch BioPlot from Database Explorer, with a molecule highlighted, select **Analyze > BioPlot – Analyze Selected Molecule**, or select **Analyze > BioPlot – Open New Analyzer Window**. From a Molecule Display Window or other Vector NTI applications, make the same selections, although of course the molecule to be analyzed will be that opened in the display window.

You can also drag a molecule from a partial Database Explorer screen or other Vector NTI applications into an empty BioPlot workspace.

Features of the BioPlot Workspace

Depending upon how BioPlot is first opened, the display window may be empty. As a new project is created or an existing BioPlot project is opened, the analysis results then appear in the display window.

If your BioPlot display window is empty, open a demo project into the workspace before you review its features. Select **File > Open**. Find the Vector NTI Suite folder. *It is probably located in Program Files\Vector NTI\Demo Projects*. Open the DNA or protein.apf file.

The BioPlot interface (Fig. 28.1) consists of a menu bar, three toolbars, and a BioPlot Display window, with shortcut menus specific for each pane.

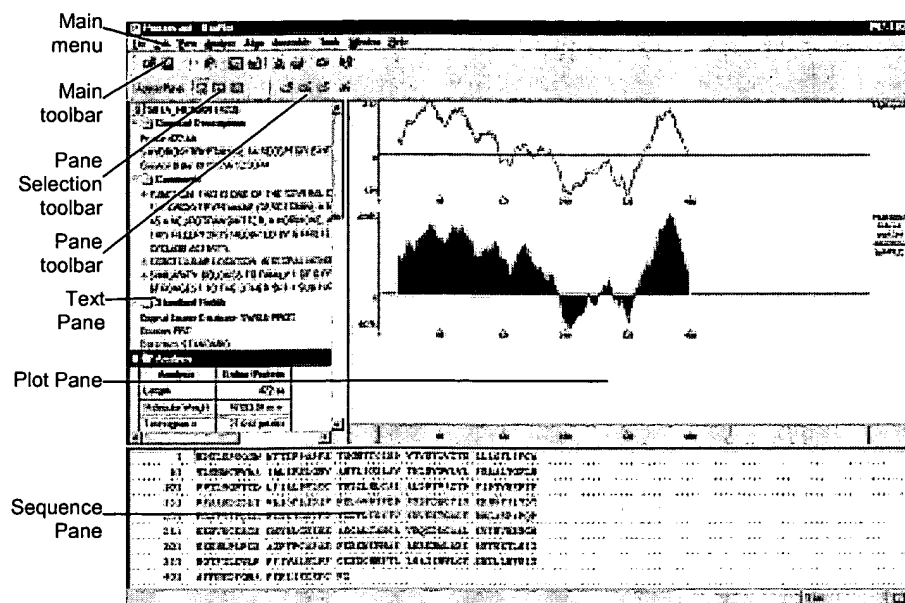





Fig. 28. 1 BioPlot interface

BioPlot has three toolbars: the Main toolbar, the Pane Selection toolbar, and the Pane toolbar. The Main Toolbar contains tools for major program functions, such as opening BioPlot analysis projects, importing molecule data from various formats, adding and deleting analyses, etc. Many toolbar commands can also be launched from the menu bar or from a shortcut menu.

All Vector NTI Suite toolbar buttons are summarized in Chapter 4. As you are introduced to various operations in this chapter, however, the toolbar buttons you will use are displayed as needed.

Each Display Window is divided into three section or panes: a Text Pane, a Plot Pane, and a Sequence Pane. Split bars allow the resizing of the panes in the display window. In BioPlot, the heavy line at the bottom or top of a graph is also a split bar. Each pane has its own scroll bars, allowing vertical and horizontal movement through the pane.

As in the other Vector NTI Suite applications, to apply a command from the main menu in a given pane (Print, Print Preview, Camera), that pane must be active. The active pane can be toggled with the Switch Panes (, , ) buttons in the Pane Selection Toolbar or by clicking anywhere in the pane you want to activate.

Elements of BioPlot Display Windows

Text Pane

To activate the Text Pane, click on the **Text Pane** button () on the Pane Selection Toolbar.

The Text Pane contains a thorough description of the analyzed molecule. The information is organized in a tree structure with the following folders:

Folder	Contents
General description	Molecule type, form (for DNA only), length, etc.
Proprietary fields	User defined fields (for molecules that came from Vector NTI)
Standard fields	GenBank/SWISS-PROT-like fields: keywords, division, original accession numbers, etc
Comment	Arbitrary text of any length associated with the molecule
References	Bibliographic references (in GenBank/SWISS-PROT format)
Feature table	List of molecule features

Table 28. 1 BioPlot Text Pane folders

The following Text Pane manipulations are outlined in Chapter 3:

- Expanding and collapsing folders
- Opening the folder shortcut menus
- Copying pane contents to the clipboard


Plot Pane

The Plot Pane consists of the plots region, a common horizontal axis, and scrollbars.

A plot consists of a vertical axis, showing minimal and maximal values of analysis results, and an individual horizontal axis, displaying either numerical positions in the sequence or residues. *Even if residues do not show initially, if you enlarge the region enough, individual residues are displayed.* The data region graphical analyses and the legend region displays the name of each analysis. To change the width of the description section, drag the thin vertical bar in the right corner of the common horizontal scale.


User Interaction Modes

BioPlot has two *modes* for working with plots.

- **Regular Mode** (default mode): In this mode, regions of data can be selected, the scale of plots can be changed, and the data section can be scrolled. In this mode, the Plot Pane is synchronized with the Sequence Pane. This means that a selected data region on the Plot Pane is displayed concurrently in the Sequence Pane and vice versa.
- In the **Plot Layout Mode**: The plots are treated as pictures that can be arranged in a number of ways, including changes in positions and the heights of the plots. To activate the Plot Layout Mode, click the **Edit Layout** button () on the Plots Pane Toolbar. This enables several edit buttons at the far right of the toolbar.


By default, plots do not overlap, but you can resize them by dragging horizontal separator bars.

Plot Layout Mode




To perform any of the following operations, BioPlot must be in the Plot Layout Mode, activated by pressing the **Plot Layout** button ()

Selecting Plots






To perform certain actions on displayed plots, you must activate Plot Layout mode, by

clicking the **Plot Layout** button () , then select one or more plots. To select a plot, click on it with the mouse. If you hold the SHIFT key down, previously selected plots stay selected; otherwise, they become unselected as you click on new selections. Selected plots can be distinguished from unselected ones by the black boxes or “handles” in the corners of the selected plot (Layout Mode only—see below).

Overlapping Plot Graphics


- If the Enable Overlapping option is turned on (with the **Enable Overlapping** button ()), one plot can be superimposed on another (drag it into position). The overlapping order can also be modified. To bring a plot to the front, use the **Bring to Front** button () on the toolbar or select **View > Bring to Top**. To send a selected plot to the back, press the **Send to Back** button () on the toolbar or select **View > Send to Back**.
- When the Enable Overlapping option is turned off, superimposed plots return to their original order.

Changing Plot Position

- If the Enable Overlapping option is on, the plot can be dragged and dropped anywhere in the Plot Pane. Click on a plot + drag the mouse. The cursor shape is changes from  to . It will be placed on top of other plots that might occupy the same position.
- If the Enable Overlapping option is off, the plot can be dropped in specific positions only. When the cursor shape changes from  to , a new position is allowed. When the cursor shape changes to , the new position is invalid.


Changing Plot Height

Plot height can be changed only for a single plot at a time.

- To modify the plot height, move the cursor to one of the handles where it changes to a two-headed arrow (). Drag the handles vertically to resize the plot.
 - If the Enable Overlapping option is on, handles appear at the top and bottom of a graph.
 - If the Enable Overlapping option is off, handles appear only at the bottom of a graph. Data selection

Regular Mode





Selecting Data

A region of data may be selected in the Regular Mode only. To select a region, press and hold the mouse button and drag the mouse horizontally over the plot. To modify the current selection either hold the SHIFT key down and click/drag in the plot area or resize the edges of the existing selection by dragging them (the cursor changes to .


By clicking outside the plot area or individual horizontal axis, BioPlot removes the selection from plot.

Selecting a region on one of the plots also changes selected regions on all plots.

Changing Scale




As in other Vector NTI Suite applications, plot analysis graphs can be resized using the Zoom buttons. The scale for all plots can be changed by using the **Zoom In** () and **Zoom Out** () buttons or by selecting **View > Zoom In** or **Zoom Out**. Increase the scale of a selected region in the Plots Pane by clicking the **Zoom to Selection** button (). *In this case, now you can read the individual residues in the Plot Pane.* To fit the entire plot into the data section, use the **Fit to Window** button () or select **View > Fit to Window**.

Editing Analysis Properties

To modify individual properties and common display properties, double-click on a plot, opening the Plot Properties dialog box. Alternatively, click on a plot once to select it, then press the **Properties** button () or choose **View > Properties** command from the menu bar.

In the Plot Properties dialog box, both the analysis parameters and the display setup for the plot can be changed. The Analysis tab contains a short description of the analysis and some analysis-dependent parameters. The Display tab changes the display attributes of the plot, such as plot type and color. It also turns the display of the individual scale for the plot on or off.

Changing Common Display Properties

The common display properties of all plots can be customized using buttons on the Plot Pane toolbar of the View menu. The **Show Vertical Axis** command/buttons () turns on/off the display of vertical axes on all plots. The **Show Legend** () and **Legend Position** () buttons turn on/off and change position of the legends for all of the plots.

Display Setup for Analyses is discussed later in this chapter.

Sequence Pane

As in Vector NTI Suite applications, in BioPlot nucleotide or amino acid sequences can be viewed, manipulated and formatted in the Sequence Pane. Regions of the sequence can be copied and pasted to the Clipboard.

Sequence Pane Elements

In addition to the sequence, the Sequence Pane displays a molecule's sequence features. Features are shown with a horizontal line above the sequence (protein features and DNA features on the direct strand) or below the sequence (DNA features on the complementary strand). Labels also show the name of the feature.

The sequence can be scrolled up or down with the Sequence Pane's scroll bar. If Sequence Pane is the active pane, standard arrow keys may be used to scroll the sequence.

Navigating the Sequence Pane Using the Keyboard

Standard keyboard keys may be used to navigate the sequence:

Key	D scription
Left	Move caret to previous position
Right	Move caret to next position
Up	Move caret position one line up
Down	Move caret position one line down
Home	Move caret position to the beginning of the current line
End	Move caret position to the end of the current line
Ctrl-PgUp	Move caret position to the beginning of sequence
Ctrl-PgDn	Move caret position to the end of sequence


Table 28. 2 Navigating a sequence using the keyboard


Selecting a Sequence Region

To select sequence regions, CLICK + DRAG the mouse across the sequence. Press SHIFT + RIGHT or LEFT ARROW to start or extend a selection.

A selected region in the Sequence Pane is concurrently displayed on the plots in the Plot Pane.

Changing Sequence Pane Properties

In BioPlot, double-strand mode is default mode for DNA/RNA sequences. To switch to a single strand display, press the  button on the pane toolbar or select **View > Double Strand**.

The length of a single line of a sequence and number of blocks per line can be adjusted as well. Press the **Setup**  button on the toolbar or select the **View > Properties** to open the Setup dialog box (Fig. 28.2):

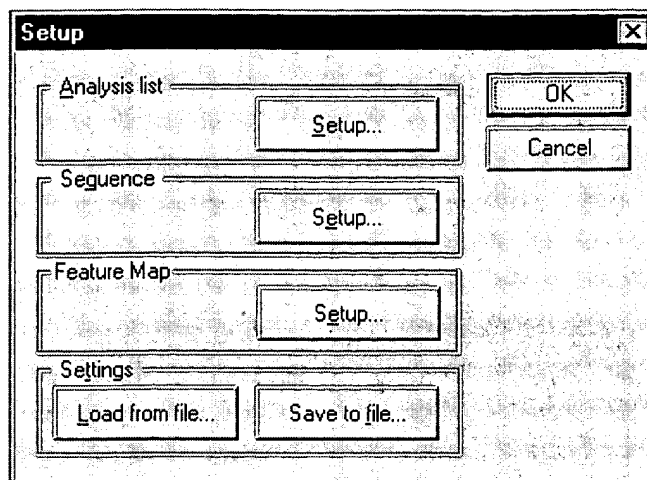


Fig. 28. 2 Setup dialog box

Press the **Setup** button for a Sequence to control sequence display (Fig. 28.3):

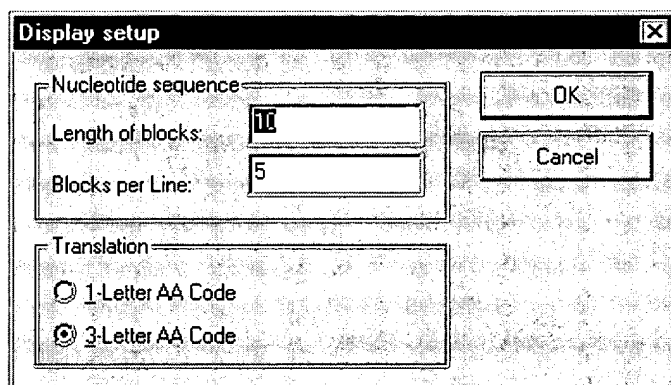


Fig. 28. 3 Sequence Display Setup dialog box

To choose what features should be shown in the Sequence Pane, click on the **Setup** button for Feature map in the Setup dialog box, opening the FMap Setup dialog box (Fig. 28.4):

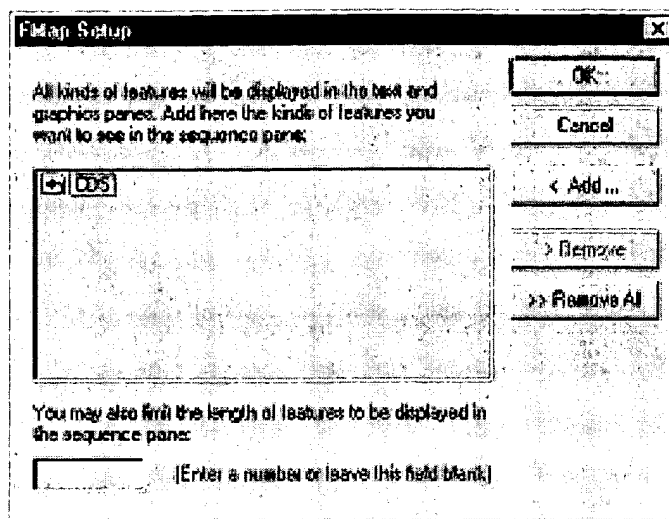


Fig. 28. 4 FMap Setup dialog box

Click the **Add** button to add features for display. Feature Map Setup is discussed in detail in Chapter 19.

Changing Sequence Character Display Attributes

Display attributes for selected sequence residues can be changed in the same way that text is formatted in a word processor using the appropriate toolbar buttons. See Chapter 3 for details.

Copying a Raw Sequence

To copy real sequence data to the Clipboard, select a sequence region and choose **Edit > Copy**. The sequence will be copied in the plain text format without formatting or line numbering. This “raw” format is suitable for pasting into other molecular biology software including programs in the Vector NTI Suite. The keystroke for the copy operation is **Ctrl+C**.

BioPlot Projects

Creating a New Project

When BioPlot is launched, a new, empty display window is opened. To create a new project, you must import molecule information from various molecule file formats. To import a molecule, select **File > Open** on the menu bar.

BioPlot reads the following file formats: GenBank and EMBL for DNA, GenPept and SWISS-PROT for proteins. BioPlot also reads ASCII Text, FASTA, and Vector NTI archives for both DNA and proteins.

In the File Open dialog box that opens, select the desired file and press **Open**. The program tries to recognize which format is being used and whether the sequence is nucleic acid (DNA/RNA) or amino acid (proteins).

If the program fails to define a file's format, it will be suggested in the following dialog box that the file be imported as a raw sequence (Fig. 28.5):

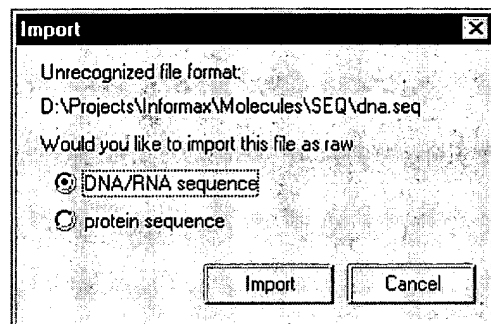



Fig. 28. 5 Importing a raw sequence

To create a new BioPlot project from Vector NTI, in Database Explorer, select a molecule, then choose **BioPlot > Analyze Select Molecule** or **BioPlot > Open New Analyzer Window**.


Opening an Existing Project

BioPlot can save a molecule and its an analysis list (with individual options) and displayed character attributes, as a single file. At any time, BioPlot can restore the contents of this file and continue to work with it. An aggregate set of molecule, options, and attributes is called a BioPlot project.

To open an existing project, choose **File > Open** or press the  button on the toolbar. In the Open dialog box, select the desired file and press **Open**. *By default, BioPlot project files have an "*.apf" file extension.* If the file is an analyzer project, information about the molecule, its analyses, and sequence display character properties (if any) appears in the appropriate panes. Another way to open an existing project is to drop the project file on the BioPlot window.

Analysis List Setup

The important part of configuring BioPlot is the selection of analyses to perform for a molecule. To add or remove any analysis from the plot, open the Analysis List Setup dialog box where you can choose from the list of available analyses.

To open the dialog box, press the **Analysis List** button  on the Main Toolbar, choose **Edit > Analysis List** on the menu bar or press the **Setup** button for Analysis List in the

Setup dialog box described previously in this chapter. This opens the Analysis List Setup dialog box (Fig. 28.6).

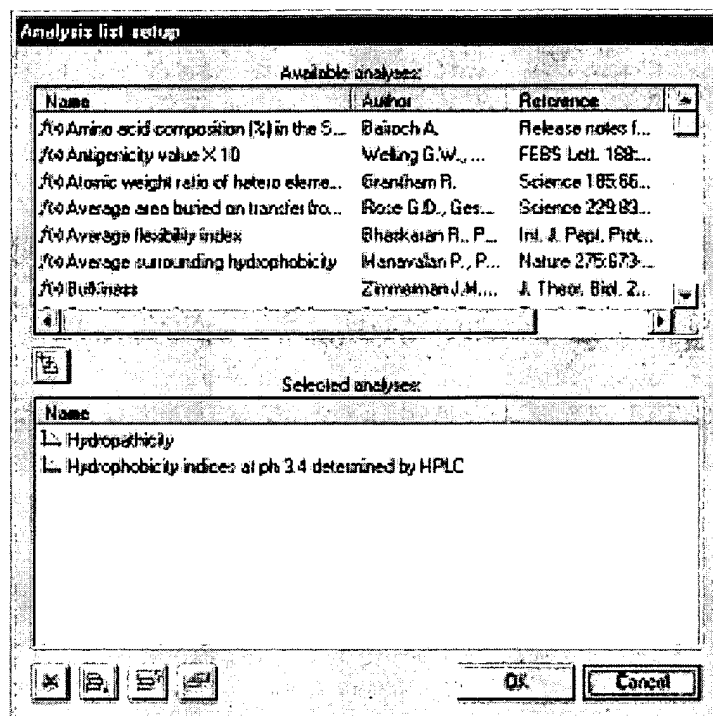






Fig. 28. 6 Analysis List Setup dialog box


The dialog box lists currently displayed analyses in the Selected Analyses box and a list of 50 predefined analyses in the Available Analyses box. *Available analyses differ for DNA/RNA and protein molecules.* Citations for each analysis are noted in the dialog box.

To add one or more analyses to the displayed analysis list, in the upper list, select the appropriate analyses and press the () button or double-click on each analysis to be added. To see the same analysis with different parameters, you can add more than one "instance" of the same analysis.


The relative position of an analysis in the Selected Analyses list determines the order in which the plots appear in the Plot Pane. The list may be rearranged by moving a selected analysis up or down by pressing () and () buttons.

Once analyses appear in the bottom box, properties of the selected analyses can be changed, analyses may be moved up and down in analysis list, and analyses may be removed from the

list. To change the properties of an analysis, select it in the list and press the () button or double-click the analysis in the list.

To remove an analysis from the displayed analysis list, select it in the analysis list and press the () button.

Analysis Properties

To change analysis parameters and choose from the various plot representation options, open the Analysis Properties dialog box by pressing the () button in the Analysis List Setup dialog box, or the same button on the Plots Pane Toolbar or by double-clicking on a plot in the Plot Pane.

The Analysis Properties dialog box has two tabs (Fig. 28.7).

1. **Analysis** tab: You can choose analysis-dependent analysis parameters. In general, every analysis has its own version of this tab for editing analysis-specific parameters, but fortunately, most protein analyses have very similar Analysis tabs. Two examples of these tabs are described later in this chapter.
2. **Datagram** tab: This is the same for all analyses. You can choose the plot type (Bar Chart, Line Chart, Boolean Chart), Threshold (the minimal relative value considered as “true” for Boolean Chart) and plot color. You can also choose to display or hide the individual horizontal axis. The effect of your choices is previewed on the tab before you choose **OK**.

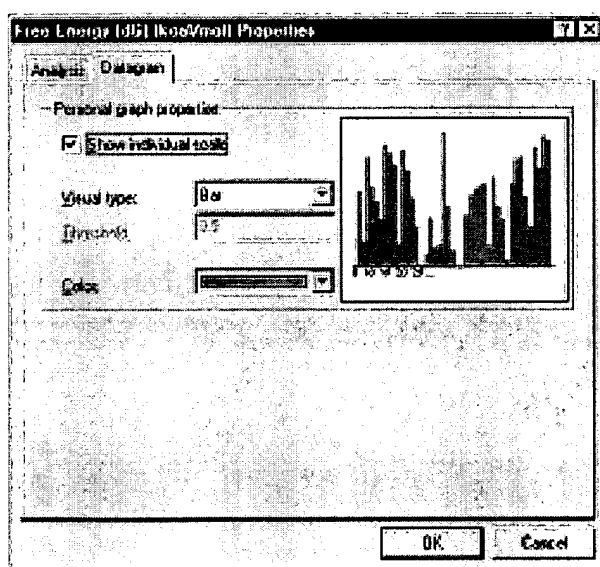


Fig. 28. 7 Analysis Properties dialog box

Other BioPlot Features

The following features can be performed in the BioPlot Display window. Details specific to BioPlot are outlined here. For more information, refer to Chapter 3.

- Printing from BioPlot
- Copying data from BioPlot
- Exporting molecule data

Analyses Descriptions

DNA/RNA Analyses

Melting temperature and free energy are calculated using the “nearest neighbors” method. Constants and algorithms used for calculation of thermodynamic parameters may be found in the sources listed in Appendix D.

The full list of DNA/RNA analyses are as follows:

- Free Energy (dG) (kcal/mol)
- Enthalpy (dS) (kcal/mol)
- Entropy (dS) (cal/K/mol)
- Melting Temperature (Thermodynamic) ©
- Melting Temperature (GC Content) ©
- GC Content (%)
- Nucleic Acid Distribution (%)
- Sequence Complexity

Protein Analyses

Nearly all protein analyses are based on ProtScale analyses. ProtScale allows the computation and representation of the profile produced by any amino acid scale on a selected protein.

An amino acid scale is defined by a numerical value assigned to each type of amino acid. The most frequently used scales are the hydrophobicity or hydrophilicity scales and the secondary structure conformational parameters scales. Many other scales exist which are based on different chemical and physical properties of the amino acids.

The full list of ProtScale analyses is as follows: *Full citations appear in Appendix B.*

- Amino acid composition (%) in the SWISS-PROT Protein Sequence data bank
- Antigenicity value X 10

- Atomic weight ratio of hetero elements in end group to C in side chain
- Average area buried on transfer from standard state to folded protein.
- Average flexibility index.
- Average surrounding hydrophobicity.
- Bulkiness
- Conformational parameter for alpha helix (computed from 29 proteins).
- Conformational parameter for alpha helix
- Conformational parameter for beta-sheet (computed from 29 proteins).
- Conformational parameter for beta-sheet.
- Conformational parameter for beta-turn (computed from 29 proteins).
- Conformational parameter for beta-turn.
- Conformational parameter for coil.
- Conformational preference for antiparallel beta strand.
- Conformational preference for parallel beta strand.
- Conformational preference for total beta strand (antiparallel+parallel).
- Free energy of transfer from inside to outside of a globular protein.
- Hydration potential (kcal/mole) at 25°C.
- Hydropathicity.
- Hydrophilicity scale derived from HPLC peptide retention times.
- Hydrophobic constants derived from HPLC peptide retention times.
- Hydrophobicity ($\Delta G_{1/2}$ cal).
- Hydrophobicity (free energy of transfer to surface in kcal/mole).
- Hydrophobicity indices at pH 3.4 determined by HPLC.
- Hydrophobicity scale (contact energy derived from 3D data).
- Hydrophobicity scale (pi-r).
- Hydrophobicity scale (pi-r).
- Hydrophobicity scale based on free energy of transfer (kcal/mole).
- Mean fractional area loss (f) (average area buried/standard state area).
- Mobilities of amino acids on chromatography paper (RF).

- Molar fraction (%) of 2001 buried residues.
- Molar fraction (%) of 3220 accessible residues.
- Molecular weight of each amino acid.
- Normalized consensus hydrophobicity scale.
- Normalized frequency for alpha helix.
- Normalized frequency for beta-sheet.
- Number of codon(s) coding for each amino acid in univerBioPlotI genetic code.
- Optimized matching hydrophobicity (OMH).
- Overall amino acid composition (%).
- Polarity (p).
- Polarity.
- Proportion of residues 95% buried (in 12 proteins).
- Recognition factors.
- Refractivity.
- Relative mutability of amino acids (Ala=100).
- Retention coefficient in HFBA.
- Retention coefficient in HPLC, pH 2.1.
- Retention coefficient in HPLC, pH 7.4.
- Retention coefficient in TFA.

ProtScale Algorithm

All of the ProtScale analyses use one algorithm:

$$D_i = \frac{\sum_{j=-W/2}^{W/2} f(S_{i+j})}{W} \quad \text{for Average Method and}$$

$$D_i = \frac{\sum_{j=-W/2}^{W/2} f(S_{i+j}) * Wh_j}{W} \quad \text{for Weight Method.}$$

where,

D_i - plot value in i^{th} position

W - window size

S_i - amino acid in i^{th} position

$f()$ - coefficient for amino acid from table of coefficients (specified for each of analysis)

Wh_j - linear weight coefficient

For the ProtScale analyses, the Analysis tab in the Analysis Setup dialog box has the following format (Fig. 28.8):

Average surrounding hydrophobicity Properties

Analysis **Datagram**

Name: Average surrounding hydrophobicity
 Author(s): Manavalan P., Ponnuswamy P.K.
 Reference: Nature 275:673-674(1978).
 Comments: Last modified 15/Mar/1996 by ELG

☒ Weighted average Minimal value: 0
☐ Average Maximal value: 5
 Window size: 5

Name	Value
W: Ala	12.97
W: Arg	11.72
W: Asn	11.42
W: Asp	10.85
W: Cys	14.63
W: Gln	11.76

OK Cancel Apply

Fig. 28. 8 ProtScale analyses: Analysis tab of the Setup dialog box

In addition to ProtScale, analyses in BioPlot include the *Sequence Complexity* analysis. *Full citation is in Appendix X.* For Sequence Complexity analysis, the Analysis tab in the Analysis Setup dialog has the following format:

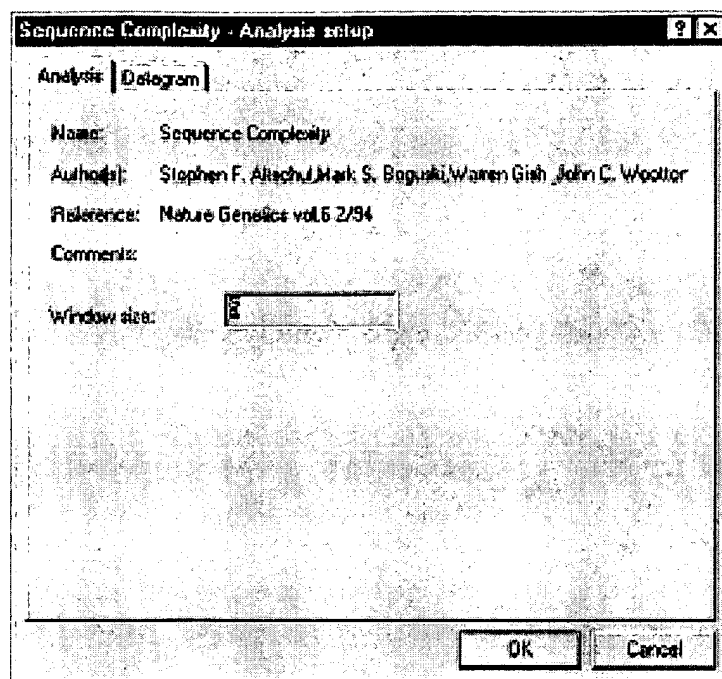


Fig. 28. 9 Sequence Complexity analysis: Analysis tab of the Analysis Setup window

Adding Custom Protein Analyses

Specific values are used in the calculation of the various protein analyses. These values differ depending on the particular analysis and are stored in small text files having a .pa2 extension. All .pa2 analysis files are stored in the Analyses subdirectory in the Vector NTI Suite directory. The name of the analysis corresponds with the name of the .pa2 file.

For example, the Bulkiness analysis is characterized in the Bulkiness.pa2 file as follows:

```
[general]
author=Zimmerman J.M., Eliezer N., Simha R.
comment=Last modified 15/Mar/1996 by ELG
reference=J. Theor. Biol. 21:170-201(1968).
unique=s30
[data]
values=11.5,14.28,12.82,11.68,13.46,14.45,13.57,3.4,13.69,21.4,21.4,15.71,16.25,19.8,
17.43,9.47,15.77,21.67,18.03,21.57
```

The [general] section describes the attributes of the analysis and the [data] section contains the list of values used for calculating the analysis. Values are listed according to the alphabetical order of the acid coefficients.

You can create your own custom protein analysis by modifying the values in one of the existing .pa2 files using a plain text editor, such as Notepad. Once modified, the new .pa2 file should be saved in the Analyses subdirectory under a new name that corresponds to the new analysis type. If BioPlot is open at the time the new .pa2 file is created, it must be closed and reopened before the new analysis is recognized.

Chapter 29 ContigExpress: Project Explorer

Introduction

ContigExpress is a program for assembling many small fragments, both text sequences and chromatograms from automated sequencers, into longer contiguous sequences or “contigs”.

Work in ContigExpress takes place in a ContigExpress Project, a file where you store fragments, their assemblies, and assembly options related to your current task. In ContigExpress, fragments can be edited directly, with the chromatograms in full view. Changes are tracked and a history is maintained. The contigs generated are then saved and managed as GenBank, EMBL or FASTA files. They can be exported into other applications of the Vector NTI Suite for further analysis or visualization. Data can also be exported to third-party tools available on the WWW.

Launching ContigExpress

To launch ContigExpress, double click on its icon in the program group or folder in which you installed Vector NTI Suite. To launch ContigExpress from Vector NTI Database Explorer, select **Assemble > ContigExpress – Assemble Selected Molecules** or **> ContigExpress – Open New Assembly Project**. You can also launch ContigExpress from most other Vector NTI Suite applications under the Assemble menu option.

Features of the ContigExpress Workspace

ContigExpress consists of three component windows:

- **CE Project Explorer:** This window is the first window you see when you launch ContigExpress. In this window you open, save, and close the projects and set the assembly options. Project Explorer is also where you can organize and assemble your fragments contained in a project.
- **Fragment Window:** This is the workspace where you work with individual fragments and chromatograms.
- **Contig Window:** Contig Window is the place where you review and edit contigs.

Fragment and Contig Windows are covered in Chapter 30.

CE Project Explorer

Project Explorer, similar to the Vector NTI Database Explorer, is central to the management and assembly of fragments into contiguous sequences. When first opened, the display window is empty. To review ContigExpress features in a complete display window, open a demo project into the workspace. Select **Project > Open Project**. Find the Vector NTI Suite folder (probably in Program Files\Vector NTI\Demo Projects.) and open DemoProject.cep.

Elements of Project Explorer

The CE Project Explorer interface (Fig. 29.1) consists of a menu bar, a toolbar, a Tree Pane on the left and a List Pane on the right side of the window.

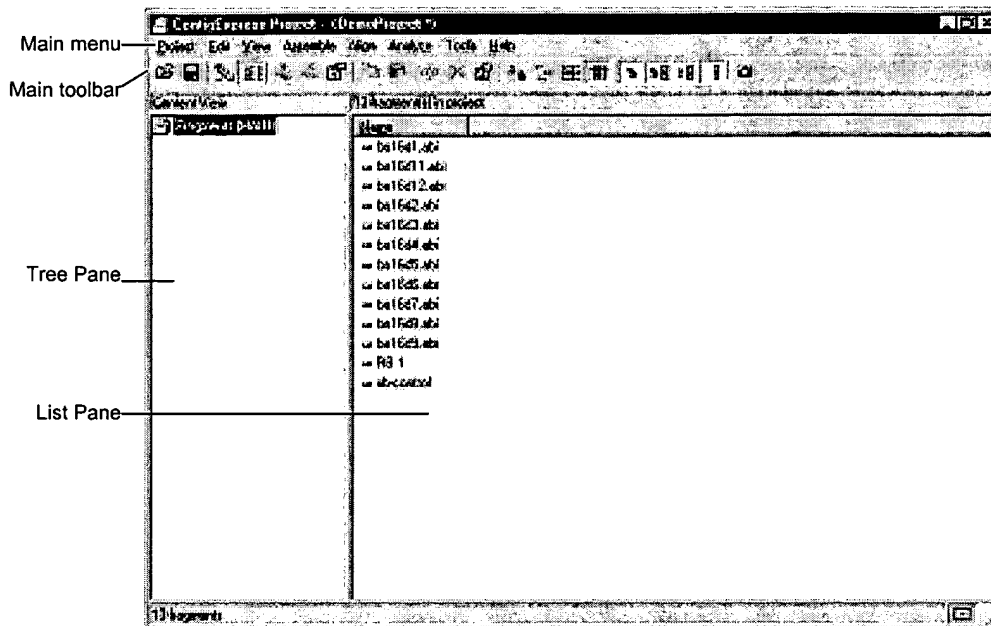


Fig. 29. 1 CE Project Explorer interface

All Vector NTI Suite toolbar buttons are summarized in Chapter 4 of this manual. As you are introduced to various operations in the chapters covering ContigExpress, however, the toolbar buttons are displayed as needed. Many toolbar commands can also be launched from the menu bar or from a shortcut menu.

The ContigExpress Project Explorer window is divided into two panes: a Tree Pane on the left and a List Pane on the right. As a new project is created or an existing ContigExpress project is opened, the fragment and Assembly folders are listed in the Tree Pane. Folders can be opened (expanded) or closed (collapsed) as in the other applications of Vector NTI.

Status bars in Project Explorer show information about the items in the Tree and List panes.

The status bar above the display panes shows the current Tree Pane view mode and the number of items contained in the selected project in the Tree Pane (Fig. 29.2).

The status bar at the bottom of the Project Explorer shows the amount and type of currently selected item(s). It can be configured to show any property of a selected single item in the List pane.

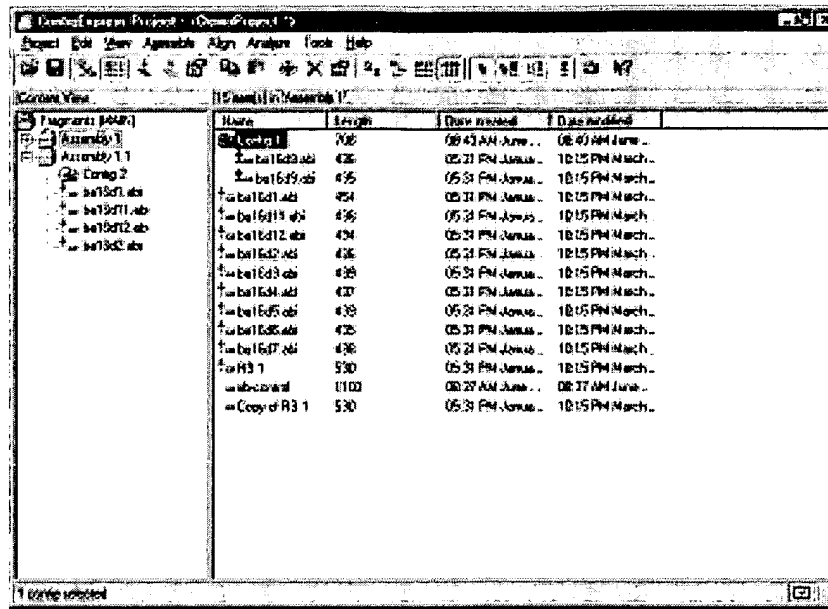







Fig. 29.2 Viewing the Status bars in Project Explorer


Tree Pane

The Tree Pane displays the general structure of your ContigExpress Project in either of two viewing modes: History or Content view. The contents of each pane are displayed and manipulated similar to other Windows applications, such as Vector NTI Database Explorer or Windows Explorer.

The following types of items can be found in the Tree pane:

-  List of all fragments in the project
-  Assembly of fragments
-  Contig
-  Fragment included in an assembly
-  Fragment not included in an assembly.

History View

Click the **History View** button () to display the Tree Pane History view (Fig. 29.3). In this mode, all assemblies contained in the project are shown in a tree that reflects their "historical" relationship (that is, the order in which they were created). In History View, the Assembly content list cannot be viewed in the Tree Pane.

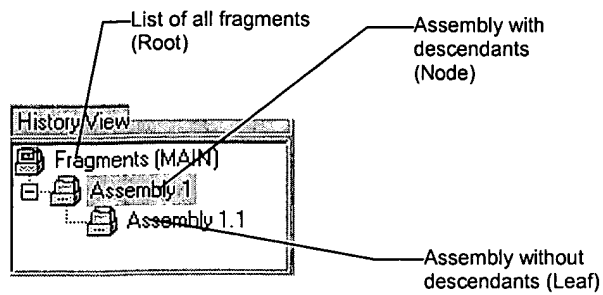



Fig. 29. 3 Tree Pane History view

The root of the tree is always the list of all fragments existing in the project. The tree nodes are assemblies that have descendant assemblies, and the leaves are the assemblies that have no descendants.

Content View

Click the **Content View** button () to display a “flat” list of all assemblies in the project without displaying their mutual relationships. In Content View, you can display in the Tree Pane the contigs in the assemblies and non-included fragments by clicking on the + button at the left of assembly name.

As in the History View, the root of the tree always list all of the fragments existing in the project. The tree nodes are the assemblies. Contigs and fragments that were attempted unsuccessfully to be included are listed under the assemblies. Fragments successfully included in the assemblies are listed inside the Contig Folders (Fig. 29.4).

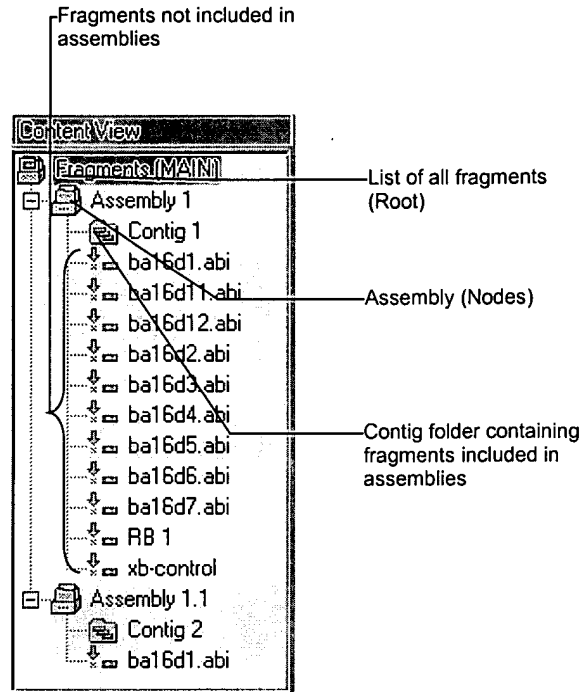


Fig. 29. 4 Contigs and fragments that were attempted unsuccessfully to be included are listed under the assemblies

The following table summarizes the contents of each of the viewing modes:

Viewing Mode	Assemblies	Contigs	Fragments in Assemblies	Fragments not Included in Assemblies
History View	Yes	Yes	No	No
Contents View	Yes	Yes	Yes	Yes

Table 29. 1 Viewing modes

List Pane

The List Pane shows the contents of an item currently selected in the Tree pane. When the Fragments (MAIN) is selected in the Tree Pane, the List Pane displays all fragments of the project (Fig. 29.5).

A fragment not included in an assembly contains no other items; therefore, nothing is shown in the List pane when it is selected in the Tree pane.

On the toolbar, the List Format group of buttons control the list display. In the first three modes, only item icons and names are displayed.

List Pane Formats





Button	Action
	View the List pane in Large Icons mode
	View the List pane in Small Icons mode
	View the List pane in List mode
	View the List pane in Details mode. In this mode, you can select the properties shown in column for each item type.

Table 29. 2 List Pane format options

List Pane Filters

The next group of buttons filters data displayed in the List pane.





Button	Action
	Enable the Show Contigs filter in List pane
	Enable the Expand Contigs filter in List pane
	Enable the Show Unassembled Fragments filter in List pane
	Enable the Show Other Fragments filter in List pane

Table 29. 3 List Pane filter options


Sorting List Pane Columns

Objects are described in the List Pane by fields in columns. Sort column objects by clicking on the property column headings (in Details mode). Reverse the order by clicking on the same column header for the second time or by selecting **View > Arrange Icons**.

General Project Explorer Operations

Opening or Creating a Project

To open a project from a disk or other location, select **Project > Open Project**, click the

Open Project button () or use Ctrl-O. You can also drag and drop a project file from Database Explorer into ContigExpress.

In the Open Project dialog box, browse the folders available on your computer and select an existing project file (extension .CEP). Select the project file and press **Open**:

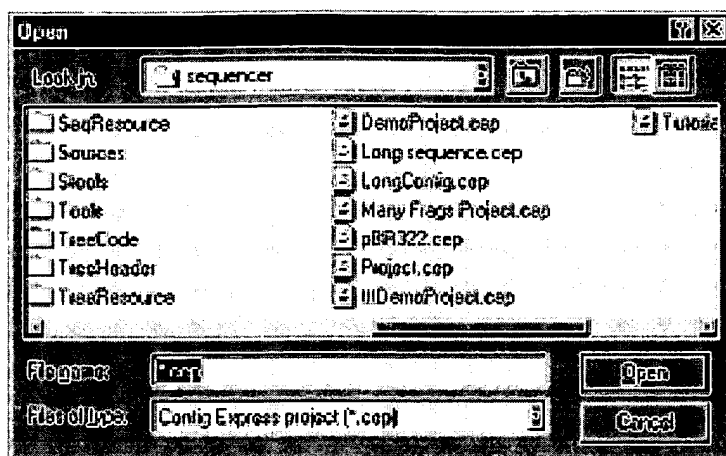


Fig. 29. 7 Open Project dialog box

If you get an error message, the selected project file probably has an invalid structure. If a project is currently open in the CE Project Explorer and it has been changed since its last save, you will be asked at this point to save the project. If you choose not to open a new project at this stage, press **Cancel**.

For your convenience, the four most recently opened projects are listed at the bottom of the Project menu option. Select **Project** > **<PreviouslyOpenedProjectName>** to open one of those listed.

After successful loading, the panes of the Project Explorer are updated to show the new project.

Import a Fragment to a Project

To import a fragment to a project, select **Project** > **Add fragments** > **From <file> ...** or from the shortcut menu, select **List of all fragments** > **Add fragments** > **From <file> .** Select the name in the Import sequence from dialog box and click **Open**.

Alternatively, select one or several files/fragments in the Windows Explorer or Vector NTI Explorer and drag them to ContigExpress Project Explorer and drop them to any of the Project Explorer panes. They will instantly be added to the project.

Files must be in one of the following formats: GenBank (*.gb), FASTA (*.txt), ABI (*.abi), EMBL (*.txt), Staden SCF (*.scf), ALF (*.alf), and plain text sequence file (*.txt).

The imported fragment(s) are added to the project and the list of all fragments is updated.

Selection Techniques

Selection techniques described here are unique to ContigExpress. General selection techniques are described in Chapter 3.

Tree Pane:

In the Tree Pane, click on an item to select it. (Only one item can be selected at a time.) Change the selection by clicking the item with the left mouse button or by moving the selection bar with the cursor buttons on the keyboard.

List Pane:

The List Pane allows multiple item selections:

- To select a range of items: SHIFT + CLICK on the first and last item in the list
- To select non-contiguous items: SHIFT + CTRL + CLICK on each item
- To select a group of items contiguous to each other in the List Pane, click anywhere in the blank area of the pane. Drag a box around the files you want to select.
- To select all the items in the List Pane, right-click on the first item in the list and choose **Select All** from the shortcut menu.


When you select an item in the Tree Pane, the contents of the List Pane are updated to show the contents of the newly selected item. The upper status bar is updated as well. The lower status bar reflects selection changes in both panes of the Project Explorer.

To Select All Items, select **Edit > Select All** or use keystrokes CTRL-A.

To invert a current selection in the List pane (that is, make all currently unselected items selected and vice versa), select **Edit > Invert Selection**.

Assembling Selected Items

To assemble a contig, select at least two items in the List Pane, and choose **Assemble >**

Assemble Selected Items, or press the **Assemble** button .

If one or several contigs are selected for assembly, the sequences of all fragments that comprise the contig(s) are used as fragment sequences for the assembly process.

Since assembling is a lengthy process, a progress dialog box (Figure 29.8) appears showing you the assembly steps. You can abort the process at any time by clicking the **Cancel** button:

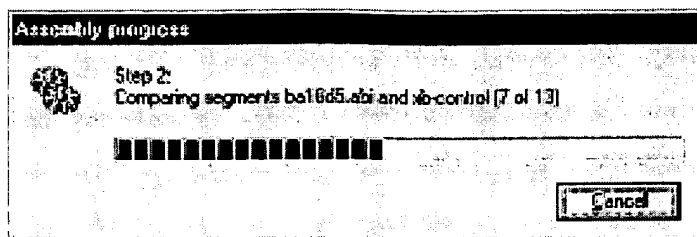



Fig. 29. 8 Assembly progress dialog box

If the assembly fails, and a contig could not be created from the selected fragments with the selected setup options, you will be informed.

If the assembly succeeds, a new "child" assembly is created inside the current assembly. The selection in the Tree Pane moves to the newly created assembly and the List Pane shows its contents.

Setting Assembly Setup Parameters

When you create or open a Contig Express project, a default set of assembly options is used for the project. To change these options, press the **Assembly Setup** button () or select **Assemble > Assembly Setup**, opening the Assembly Setup dialog box (Fig. 29.9):

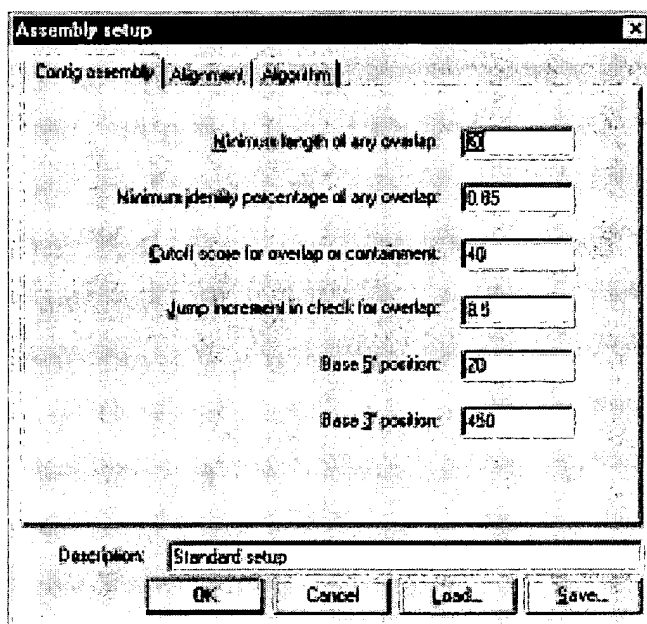


Fig. 29. 9 Assembly Setup dialog box

Contig Assembly

On the **Contig Assembly** tab, you can change or define the following parameters:

Contig Assembly Parameters	
Minimum length of any overlap (valid values: 0 - 100)	In the initial assembly, any overlap must be of this length
Minimum identity percentage of any overlap (valid values: 0.0 - 1.0)	Any overlap/containment must be of identity percentage of this setting
Cutoff score for overlap or containment (valid values: 30 - 50)	Two contigs are merged if the score of the overlapping alignment is at least this cutoff score. This value is chosen according to the value for score of a match.
Jump increment in check for overlap (valid values: 0.0 - 10.0)	This parameter defines necessary conditions for overlap or containment. Those conditions are used to quickly reject pairs of fragments that could not possibly have an overlap or containment relationship. The dynamic programming algorithm is only applied to pairs of fragments that pass the screening. A large value for jump increment means stringent conditions, where the value for jump increment is a floating point number at least 8.0.
Base 5' position (valid values: 0 - 99)	Base 5' and Base 3' are fragment positions such that the 5' end between base 1 and Base 5', and the 3' end after Base 3' are of high sequencing error rates, say more than 5%. For mismatches and indels occurring in the two ends, light penalties are used. For example, if you know that the sequencing errors often occur before some base at the beginning of the fragment, and after some base at the end of the fragment, you may enter that base numbers as Base 5' and Base 3' position.
Base 3' position (valid values: 100 - 100000)	

Table 29. 4 Contig Assembly Parameters

Alignment

On the **Alignment** tab (Fig. 29.10), you can define parameters for the alignments generated between fragments in the creation of new contigs.

Assembly setup

Config assembly | Alignment | Algorithm

Score of a match: 18

Score of ambiguous match: 1

Score of a mismatch: -6

Light score of a mismatch: -3

Gap open penalty: 0

Gap extension penalty: 4

Light gap extension penalty: 2

Description: Standard setup

OK Cancel Load... Save...

Fig. 29. 10 Alignment tab of Assembly Setup dialog box

Two specially chosen sets of substitution scores and indel penalties are used by the dynamic programming algorithm: a heavy set for regions of low sequencing error rates and a light set for fragment ends of high sequencing error rates. These scores are for the heavy set (that is, after Base 5' and before Base 3' positions).

Alignment Parameters	
Score of a match (valid values: 0 - 5)	Score assigned matching nucleotides
Score of ambiguous match (valid values: 0 - 4)	Score assigned to a match that includes an ambiguous residue
Score of a mismatch (valid values: -10 - 0)	Score assigned to mismatched nucleotides in regions of low sequencing error rates
Light score of a mismatch	Score assigned to mismatches in fragments ends (regions of high sequencing error rates)
Gap open penalty (valid values: 0 - 5)	The penalty for the first residue in a gap
Gap extension penalty (valid values: 0 - 5)	The penalty for additional residues in a gap in regions of low sequencing error rates

<i>Alignment Parameters</i>	
Light gap extension penalty	The penalty for additional residues in a gap in fragments ends (regions of high sequencing error rates)

Table 29. 5 Alignment Parameters

In the description field on each tab, enter text to name and distinguish the different sets of parameters.

On either of these tabs, if you enter an invalid value (outside the permissible ranges) and press any button except **Load...** and **Cancel** or if you change the current tab, you will be asked to correct your action. ContigExpress informs you about the correct ranges and moves the cursor to the offending field.

Algorithm

On the **Algorithm** tab (Fig. 29.11), you can choose the algorithm you would like ContigExpress to use for contig assembly. There are two algorithms to choose from, Pairwise Assembly and Linear Assembly.

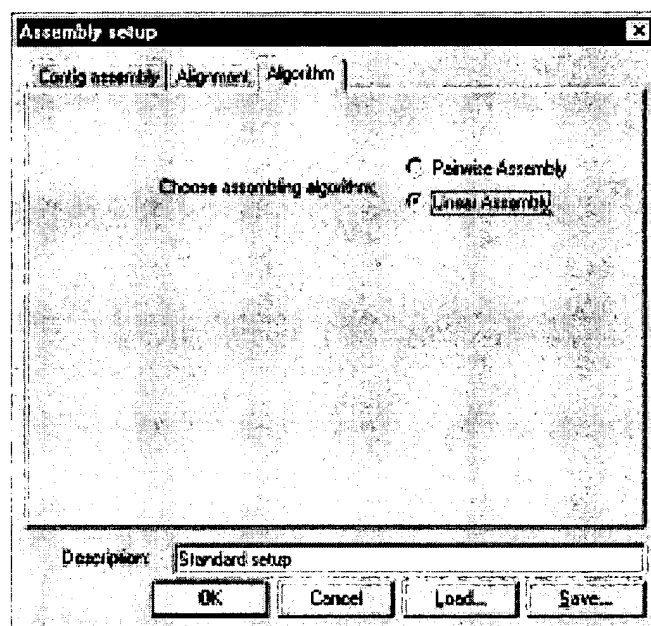


Fig. 29. 11 Algorithm tab of Assembly Setup dialog box

Note: the Linear Assembly algorithm uses the Minimum Length of Any Overlap parameter on the **Contig assembly** tab for its calculations. Other parameter settings on the **Contig assembly** and **Alignment** tabs do not affect this algorithm.

Features of the Pairwise and Linear Assembly algorithms are outlined in the following table:

<i>Algorithm</i>	
Pairwise Assembly	<ul style="list-style-type: none"> • best for assembling ten or fewer fragments • faster initialization • lower memory use • may be faster for small numbers of fragments • more stringent default conditions • user can set more assembly parameters; allows higher degree of assembly customization
Linear Assembly	<ul style="list-style-type: none"> • best for assembling 11 or more fragments • slower initialization • higher memory use • faster assembly of large number of fragments • less stringent default conditions; may allow assembly of fragments with smaller regions of overlap • fewer parameters to set; only Minimum length of any overlap parameter is used

Table 29. 6 Features of the Pairwise and Linear Assembly algorithms

Press the **Load** and **Save** buttons to read and save the parameters set in Assembly Setup. The Open or Save dialog boxes open respectively where you can select a parameter set to be loaded or a file where the parameter set will be saved. After loading a parameter set file, all fields of the dialog box are updated.

Press **OK** to validate the entered parameters; they will apply to all future assemblies in the project. Press **Cancel** to abort the action.

Editing an Item’s Data

To edit data about an item, select the item, then select **Edit** on the shortcut menu. In the Edit dialog box (Fig. 29.12) you can change the name, description and comments of the item:

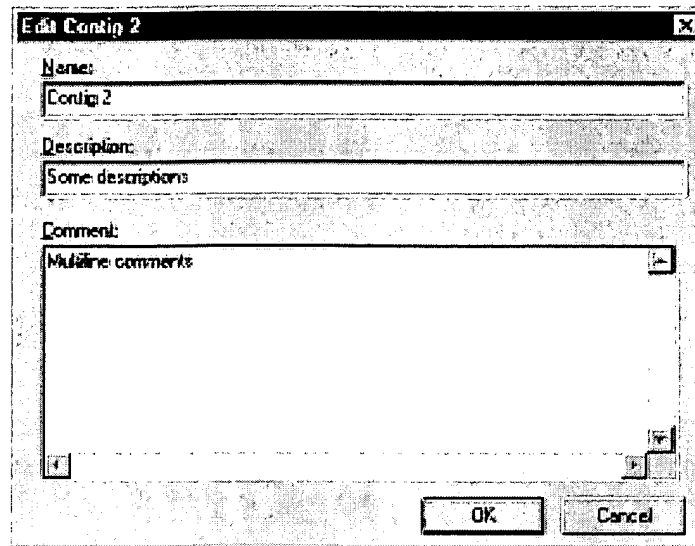



Fig. 29. 12 Edit dialog box

Click **OK** to confirm the changes; all instances of the item throughout ContigExpress are updated to reflect the changes. Click **Cancel** to abort the action.

Editing Project Properties

To edit a project's properties in ContigExpress, press the **Properties** button () or select **Edit > Properties**. The corresponding keystroke is ALT-ENTER. This opens the Properties dialog box (Fig.29.13) where you can review the properties of the selected item(s).

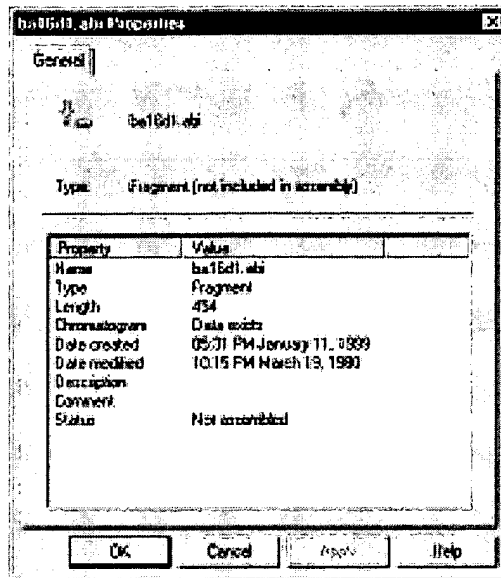


Fig. 29. 13 Properties dialog box

For several selected items, the only property shown is the number of selected items (Fig. 29.14):

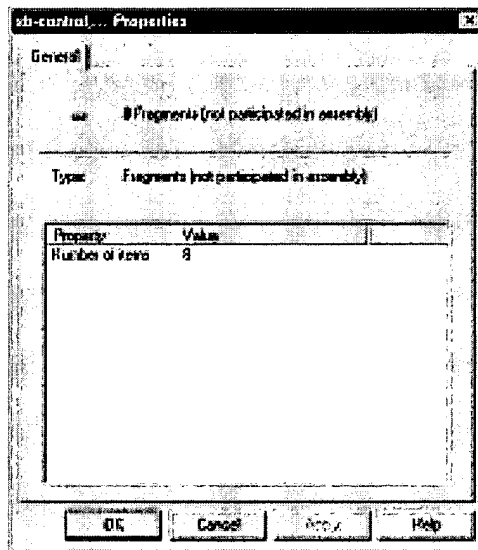


Fig. 29. 14 Number of selected items property


Open Contig(s)/Fragment(s)

To view or edit one or more contigs or fragments, double-click the item or select **Open** in its shortcut menu.

The Contig or Fragment Windows for each of the selected items is opened.

For further information on how to work in the Workspace, Contig, and Fragment Windows see the respective chapters of this manual.

Dismiss Assembly


Dismissing an assembly deletes a selected assembly from the open project. When an assembly item is selected in the Tree Pane or when the List Pane is active and shows the contents of an assembly, click the **Dismiss Assembly** button () or select **Edit > Dismiss Assembly** or the comparable option on the shortcut menu. If the assembly has “child” assemblies, they will be dismissed as well. The Project Explorer display window is updated to reflect the changes.

Dissolve Contig


Dissolving a contig removes the selected contig from the assembly, but leaves the rest of the assembly intact. To execute the Dissolve Contig command, right-click on the contig in the List Pane of the CE Project Window and choose Dissolve Contig from the shortcut menu. Once the contig has been dissolved, the CE Project Explorer display window is updated to reflect the change.

Delete Contig(s)/fragment(s) from Project

To delete selected item(s) from the project, select the item in the List Pane and click the

Delete button () , select **Edit > Delete from Project** or press the keyboard DELETE button. If any of the items to be deleted are included as a fragment in any assembly, you will be warned that all assemblies (and their child assemblies) that contain the item will be dismissed. You can confirm or cancel the action.

Rename Item

To rename a project item, select the item and press the **Rename** button () , select **Edit > Rename** or single click inside the name of the item to be renamed. Enter the new name in the enabled text box. If the item that has been renamed is used elsewhere in the project, all instances of the item will be renamed.

Operations Changing the Project Items

A fragment with the same sequence as a contig’s consensus and the same name as the original contig can be created and added to the project. To convert an existing contig to a fragment, select **Project > Convert Contig to Fragment** on the menu bar or select **Convert Contig to Fragment** on the shortcut menu.

Trimming Fragments

Trimming fragments is often performed to remove unreliable (ambiguous) residues. To trim one or more fragments selected in the Project Explorer List Pane, select **Edit > Trim Selected Fragments...** The Fragment Trimmer dialog box opens, listing all of the selected fragments and suggested operations. *See Fig. 31.1 and its related descriptions.* Click **OK** to proceed with trimming the fragment(s). If no action is suggested, the **OK** button is disabled.

Notes on Fragment Trimming:

- This group of actions is used to trim the selected fragments according to various criteria.
- Because “trimming” applies only to fragments, if contigs are selected, they will be skipped.
- If a fragment selected for trimming is currently open in its Fragment Window, initiating the trimming mode in Project Explorer automatically forces the fragment into the read-only mode in the Fragment Window to avoid possible inconsistencies in the project after a successful trimming.
- If any of these fragments were already changed in their respective windows, select **Yes** to save the changes or **No** to lose the changes or **Cancel** to skip the fragment in question.

Project Explorer reflects the changes if fragments are trimmed. If any of the changed fragments are open in Fragment Windows, the respective windows are updated as well.

For further information on the Fragment Trimmer dialog box, including modifying trimming settings, refer to chapter 31 of this manual.

Calling Secondary Peaks for Fragments

If conflicting residues appear on a chromatogram, you can alter the nucleotides as you wish. With one or more fragments selected in the Project Explorer List Pane, select **Edit > Call Secondary Peaks...** for Selected Fragments. The Call Secondary Peaks dialog box opens (Fig. 31.7), listing all selected fragments will be presented to the user. Click **OK** to continue.

Notes on Calling Secondary Peaks:

- Because this operation applies only to fragments, if contigs are selected, they will be skipped.
- If a fragment selected for secondary peak search is currently open in its Fragment Window, initiating this operation in Project Explorer automatically forces the fragment into the read-only mode in the Fragment Window to avoid possible inconsistencies in the project after a successful search.
- If any of these fragments were already changed in their respective windows, select **Yes** to save the changes or **No** to lose the changes or **Cancel** to skip the fragment in question.

For further information on the Call Secondary Peaks dialog box, refer to Chapter 31 of this manual.

Make Reverse Complement

To generate reverse complement copies of selected contigs and fragments from the Project Explorer List Pane, select the item and choose **Make reverse complement** from the shortcut menu. If a contig is selected for this action, it is internally converted to a fragment and then the action will be applied to it.

The reverse complement copies of the selected items are added to the project with names **Copy of <name>**, derived from the names of the original items. The new item appears in the List Pane.

Generate an Assembly Summary

To write a text file with an assembly summary, select an assembly in the Tree Pane, and select **Assembly Summary...** on the shortcut menu.

In the Write Assembly Summary dialog box, enter a file name for the summary and press **Save**. If the file with this name already exists, you can overwrite the file. Press **Cancel** to abort the action.

Operations in Project Explorer

To review or modify viewing options in Project Explorer, select **View > Options**, or when the Explorer is in Details mode, click on the property column headings. This opens the Options dialog box with several tabs (Fig. 29.15). **Note:** the Options settings on each of the tabs correspond to the item type selected in the Tree Pane when you open this Options box.

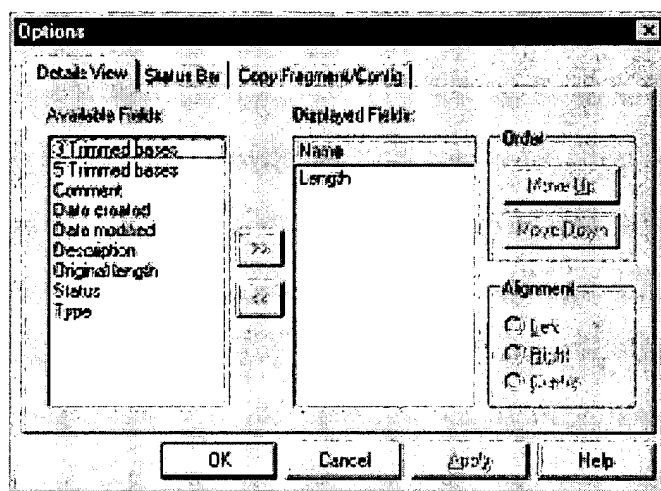


Fig. 29. 15 Options dialog box

The **Details View** tab controls the column displayed in the List Pane Details View mode. Select the desired property names in the Available Fields box and move it to the Displayed Fields box for List Pane display. The order of column display in the List pane reflects the descending order of the list in Displayed Fields. To alter the order, select a field and click the **Move Up** or **Move Down** button. To alter the text alignment for the selected item type, select the appropriate radio button.

The second of the Option tabs is Status Bar (Fig. 29.16).

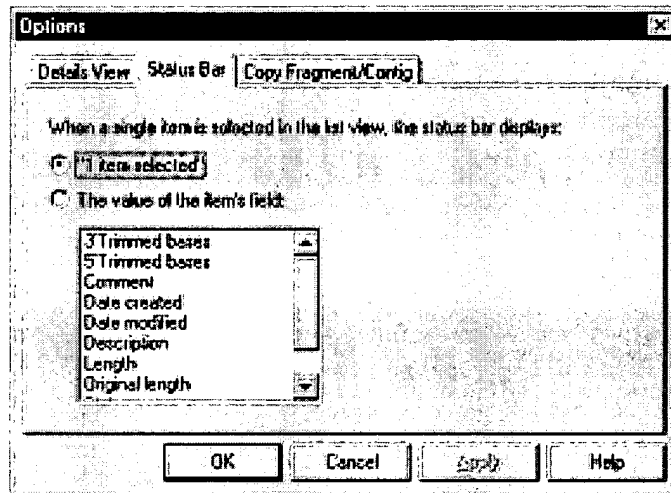


Fig. 29. 16 Status Bar tab of Options dialog box

This dialog tab allows you to choose the type of information displayed in the status bar when there is a single selected item in the List Pane. You can designate one item property to be displayed or just use the **1 item selected** option.

The third of the Option tabs is **Copy Fragment/Contig** (Fig. 29.17). The option is common for both item types:

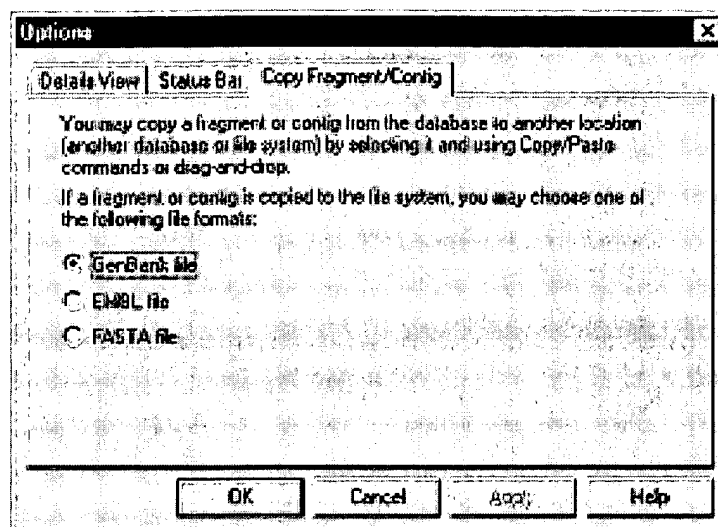




Fig. 29. 17 Copy Fragment/Contig tab of Options dialog box

Here you can define the file format used when you drag an item outside of the Project Explorer or copy it to the Windows Clipboard.

Copying Selected Fragments or Contigs

In Project Explorer, to copy a fragment or a contig select it and press the **Copy** button



(). In the List Pane, press the **Paste** button (). The copied object is added to the list of objects, automatically named "Copy of <selected object>".

Printing

You can print the contents of the Tree Pane in the CE Project Explorer window (Fig. 29.18). You can print the contents of the Tree Pane in the CE Project Explorer window. To review what the printout of the Tree pane will look like before it is printed, select **Project > Print Preview**:

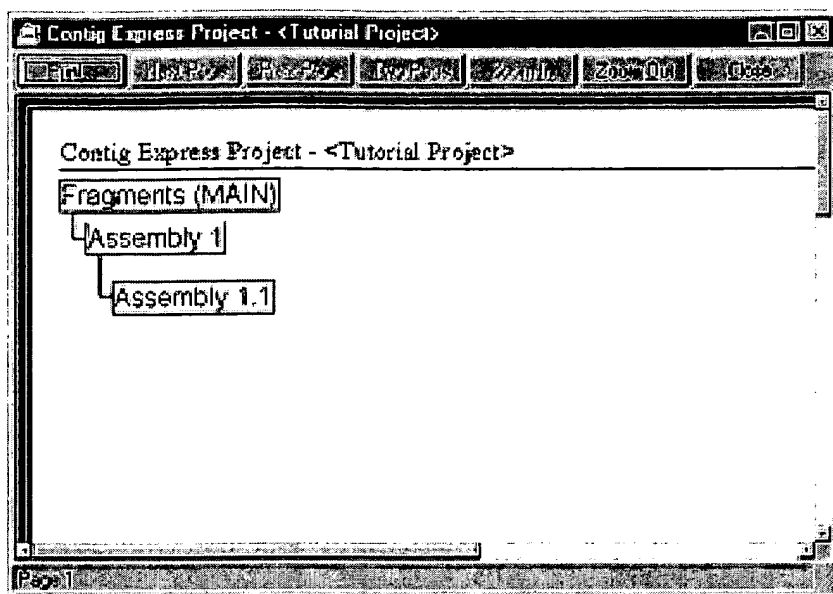


Fig. 29. 18

In the print preview dialog box, you can resize the displayed output, send it to the printer, or press **Close** to return to the Project Explorer. To define your printer options, select **Project > Print Setup**. A standard Window Printer Setup dialog box opens.

To print a copy of the Tree Pane data, activate the Tree Pane. *The Tree pane is always printed in the History view mode with the tree completely expanded.* Select **Project > Print**. In the Print dialog box, click **OK** to print or **Cancel** to abort the action.

Exporting Items from the Project


To export contigs or fragments selected in the Project Explorer List Pane, select **Project > Export Item > To <file>** or select **Export Contig/Fragment > To <file>** on the shortcut menu. ContigExpress can export items in the following formats: GenBank (*.gb), FASTA (*.txt), EMBL (*.txt), and plain text sequence file (*.txt).

In the Export <name> To dialog box, select or enter a file name and destination for the exported item. Press **Save** to export the item to the entered file. You can overwrite an existing file or press **Cancel** to abort the action.

Alternatively, you can select one or several contigs or fragments in the List pane and drag and drop them to Windows Explorer or Vector NTI Explorer. The export format for dragged and dropped files is defined by the setting on the options tab under **View > Options > Copy Fragment/Contig page**.

You can return to other Vector NTI Suite programs or send ContigExpress data to AlignX by selecting **Align > <AlignX options>** and to BioPlot by selecting **Analyze > BioPlot – Analyze Selected Molecule**.

Saving and Closing a Project

To Save a Project to disk, click the **Save** button () , select **Project > Save**, or use the keystroke CTRL-S. Enter a file name and destination in the Save As dialog box. After the project is successfully saved, the Save toolbar button and menu item are disabled until you make any changes in the project.

To close a Project, select **Project > Close Project**. To save any changes, click **Yes** in the dialog box that opens. If you select **No**, all changes are lost.

Exit Project Explorer

To exit Project Explorer, select **Project > Exit**, **Project > Close Project**, or use the keyboard shortcut, Alt-F4. This operation closes the ContigExpress application. When you select this action, the Project Explorer first closes the current project, giving you the option of saving any changes.

Chapter 30 ContigExpress: Fragment and Contig Windows

Introduction

ContigExpress offers two workspaces where you can work with fragments or contigs. Fragment Viewer allows you to work with individual fragments. Contig Viewer allows you to work with contigs or the fragments comprising them. To open fragments or contigs in their respective viewers, select the item in Project Explorer and double-click on it or select **Open** from the shortcut menu. Depending upon the item from which it was launched, a Fragment Window or a Contig Window opens. This section reviews elements common to both types of viewer windows.

Features of the ContigExpress Workspace

These workspace windows have many of the same features as the other applications of the Vector NTI Suite: a menu bar, toolbars, and three panes and a status bar.

All Vector NTI Suite toolbar buttons are summarized in Chapter 4 of this manual. As you are introduced to various operations in this chapter, however, the toolbar buttons are displayed as needed. Many toolbar commands can also be launched from the menu bar or from a shortcut menu.

The windows are organized and managed like in any Windows application. Several windows can be opened in one workspace (Fig. 30.1).

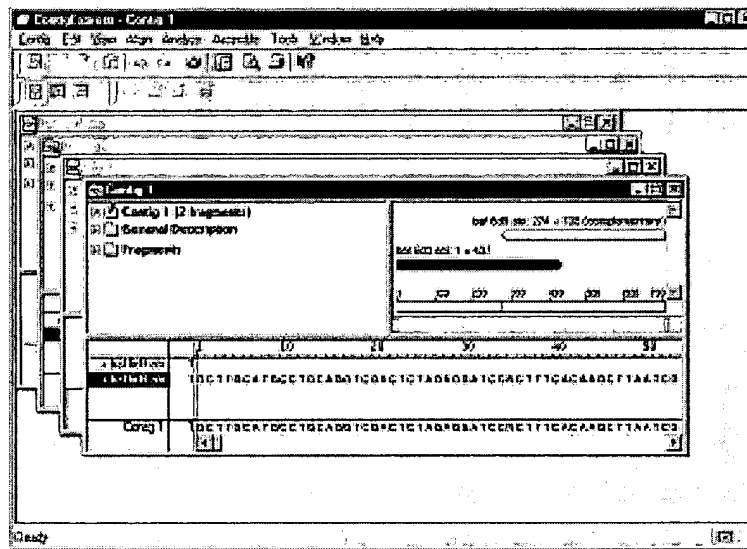


Fig. 30.1 Several Fragment Viewer windows or Contig Viewer windows can be opened in one workspace

Fragment and Contig windows are divided into three panes. Split bars allow re-sizing the three panes. Scroll bars appear when the contents of a pane exceed the viewing area, facilitating easier viewing of pane contents.

Switching Between Panes

As in the other Vector NTI Suite applications, to apply a command from the main in a given pane that pane must be active. The active pane can be toggled with the Panes buttons on the Pane Selection Toolbar or by clicking anywhere in the pane you wish to activate. The keyboard strokes are F6 (cycling clockwise through the panes) and SHIFT + F6 (cycling counter clockwise).

Status Bar

The Status bar shows menu item help and information about the caret and selection positions in the active window (Fig. 30.2).

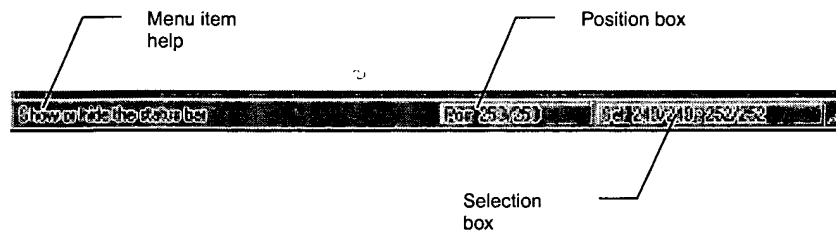


Fig. 30. 2 Status bar


The menu item help on the Status Bar is a short description of a selected menu item.

The Position Box shows the current caret position if relevant. In the Fragment Window, you can position the caret using this box. As with selection positions, because the item can contain gaps, the position is shown with gaps in the form: Counted/Not Counted.

The Selection Box displays the start and end positions of the item is selected in the active window. Because the item can contain gaps, the positions are shown with gaps both counted and not counted in the form: Counted/Not Counted.

General User Actions

Finding a Feature in Other Panes

A feature selected in a Feature Table folder in the Text Pane can be concurrently selected in both other panes. Select the feature, then press the **Find** button () on the Pane Toolbar or select Find on the shortcut menu opened from a folder.

Note: Because some features are circular, they bypass the endpoints of the fragment. Since ContigExpress does not support circular selections, such a feature will not be correctly located. The program warns you in such cases.

Editing Fragment or Contig Properties

To edit some of a selected contig or fragment's properties from the Text Pane, double-click on the first line with the fragment or contig name or select **Edit** from the associated shortcut menu.

In the Edit dialog box (Fig. 30.3), change the name, description or comments:

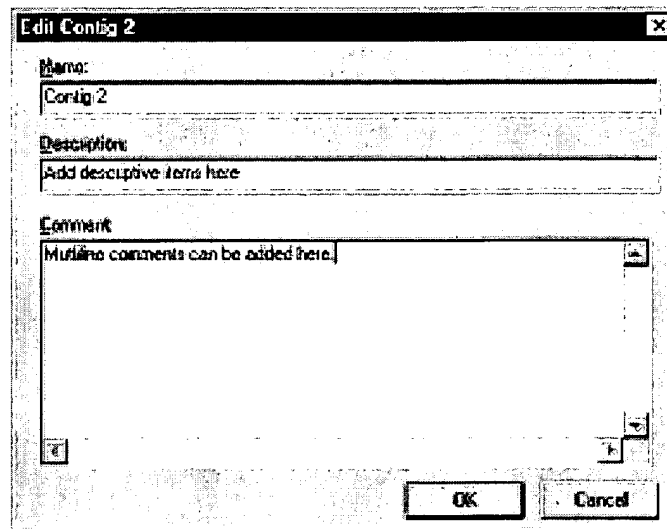



Fig. 30. 3 Edit dialog box

Click **OK** to confirm the changes or **Cancel** to abort the action.

After selecting **OK**, every instance of the fragment throughout ContigExpress is updated to reflect the changes.

Saving a Current Window

To save a changed item in a window to the project, select **Contig or Fragment > Save** or click the **Save** button (). The keystroke is CTRL-S.

If a change affects other project items, Project Explorer makes the necessary changes, and updates its display and the currently opened windows, if needed.

Closing a Current Window

To close the current window, select **Contig or Fragment > Close** or use the keystroke CTRL-F4

If the item in the window you are closing has not been saved since its last change, you can confirm saving the changes in the dialog box that opens.


Closing All Windows

To close all open windows (thus closing the Workspace), select: Contig or Fragment > Close All or use the keystroke ALT-F4.

Selecting All Data

To select all data in the current pane, select **Edit > Select all** or select **Select All** on the shortcut menu.

Deleting data

To delete a data selection from an item in the active pane, select **Edit > Delete** or press the DELETE key on the keyboard. If no selection is currently defined, one nucleotide is deleted at the caret position with this action. (If you delete in error, click on the **Undo** button ()).

The following features can be performed in ContigExpress windows. Details are given in Chapter 3.

- Resizing the panes with the split bars.
- Scrolling data inside the panes using scroll bars and arrow keys
- Opening and closing Text Pane folders
- Opening shortcut menus
- Copying selected molecule and fragments
- Copying screen and pane contents
- Pasting files and text
- Exporting data to other Vector NTI applications or external programs
- Printing from ContigExpress windows

External Tools Usage

ContigExpress is integrated with the other components of the Vector NTI suite, making it easy to launch Vector NTI, AlignX and BioPlot from ContigExpress and to easily exchange data with them. It can also easily export data to third-party tools available on the WWW.

To invoke the tools, select **Analyze, Align or Tools > <tool>**. The tools listed in these menus will work with the currently selected items.

For more information on the External Tools, refer to other chapters in the Vector NTI documentation.

Fragment Window

Launching a Fragment Window

To open a Fragment Window, select a fragment in CE Project Explorer and double-click on it or select **Open** on its shortcut menu.

Features of a Fragment Window

As in the other applications of the Vector NTI Suite, the ContigExpress Fragment window (Fig. 30.4) consists of a menu bar, two toolbars and is divided into three panes: Text Pane, Sequence Pane and Chromatogram Pane. A shortcut menu associated with specific folders or objects can be opened with a right click while the cursor is on the object. Toolbar buttons are displayed in this section as needed. All of the toolbar buttons are described in Chapter 4.

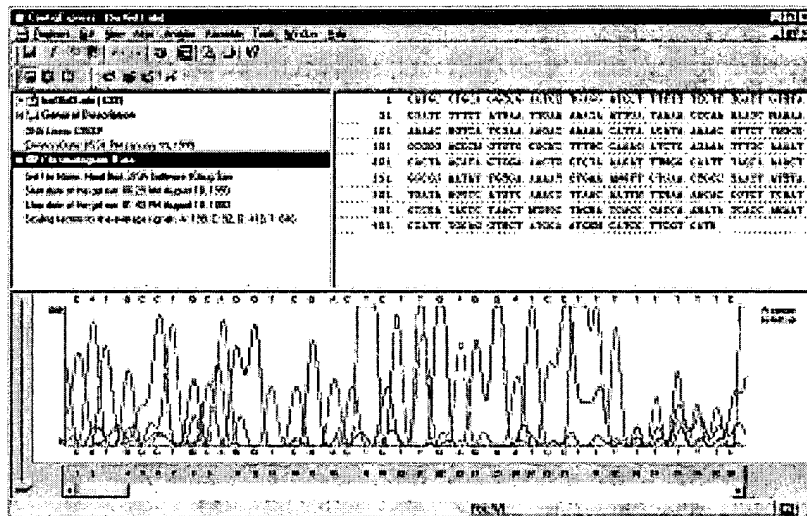





Fig. 30. 4 ContigExpress Fragment window

As in the other Vector NTI Suite applications, to apply a command from the Main Menu in a given pane, that pane must be active. The active pane can be toggled with the Switch Pane

buttons (, , ) in the Pane Selection Toolbar or by clicking in the pane you want to activate. You can also activate the panes with the F6 and SHIFT-F6 keyboard buttons.

Text Pane

The Fragment Window Text Pane contains a thorough description of the fragment, including length, date of creation, and chromatogram data.

Text Pane manipulations are outlined in the previous chapter and are also summarized in chapter 3.

The Text Pane data is divided into a hierarchy of folders and subfolder. A Text Pane can contain the following folders:

Folder	Contents
General description	Molecule type, form (for DNA only), length, etc.
Proprietary fields	User defined fields (for molecules which came from Vector NTI)
Standard fields	GenBank/SWISS-PROT-like fields: keywords, division, original accession numbers, etc
Comment	Arbitrary text of any length associated with the molecule
References	Bibliographic references (in GenBank/SWISS-PROT format)
Feature table	List of molecule features
Chromatogram data (if available)	Data imported from fragment file processed by sequencing machine (ABI files, for example)

Table 30. 1 Fragment Window Text Pane folders

Sequence Pane

The Sequence Pane shows the nucleotide sequence of the fragment with feature signals. In this pane, the nucleotide sequence can be viewed, edited and manipulated and the sequence display can be formatted. Any changes are immediately reflected in the Chromatogram Pane. Regions of the sequence can be copied and pasted to/from the Clipboard. For more details, refer to Chapter 3.

In addition to the sequence, the Sequence Pane displays a molecule's nucleotide sequence features. Features are shown with a horizontal line above the sequence (direct strand features) or below the sequence (complementary strand features) together with labels naming the feature.

Navigating the Sequence Pane Using the Keyboard

The text cursor in the Sequence Pane becomes an I-beam, also called a caret. Pause the cursor of the sequence for a few seconds to display a popup label defining the exact position of the cursor. The caret position or selection position is always indicated on the status bar.

Standard keyboard keys may be used to navigate the sequence and position the caret:

Key	Description
Left	Move caret to previous position
Right	Move caret to next position
Up	Move caret position one line up

<i>K y</i>	<i>Description</i>
Down	Move caret position one line down
Home	Move caret position to the beginning of the current line
End	Move caret position to the end of the current line
Ctrl-PgUp	Move caret position to the beginning of sequence
Ctrl-PgDn	Move caret position to the end of sequence

Table 30. 2 Navigating the Sequence Pane using the keyboard

Another way to define an exact caret position is to press the Set Caret Position box on the status bar. In the Set Caret Position dialog box that opens, enter the caret position and click **OK**.

Finding Sequence Fragments

To search for a specific sequence in the Sequence Pane, activate the pane, then press the **Find** button (F3) on the toolbar, or select **Edit > Find**.

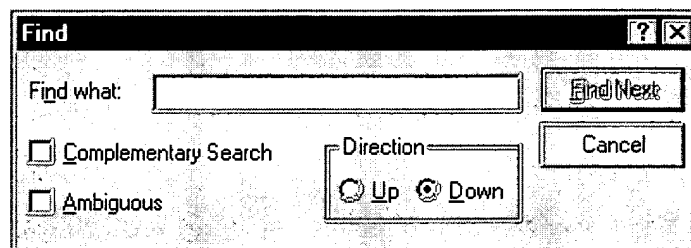




Fig. 30. 5 Find dialog box

In the Find dialog box (Fig. 30.5), enter the sequence in the text box, with criteria for the search, such as the strand to search, (direct or complementary), allow ambiguous symbols and the search direction from the current caret position. If the matching sequence is found, ContigExpress selects it and positions both the Sequence Pane and Chromatogram Pane to make it visible. If the sequence is not found, you are informed and the caret remains in its current position.

To display ORFs in the sequence, activate the sequence pane and choose **View > Show ORFs** from the menu or press the **Show ORFs** button on the toolbar. If the sequence display is double-stranded, ORFs for both strands are displayed; if the sequence is single-stranded, ORFs for the direct strand only are shown. For details on displaying ORFs in the Sequence Pane, see Chapter 17.

To search for ambiguous symbols quickly, press the **Find Previous Ambiguous**  or **Find Next Ambiguous**  buttons on the toolbar. Alternatively, you may choose corresponding commands from the View menu.

For the following operations in the Sequence Pane, refer to Chapter 3:

- Changing Sequence Pane Properties
- Changing text display attributes
- Translating sequences

Chromatogram Pane

The Chromatogram Pane displays

- the chromatogram of the fragment if it was initially provided in the original imported file used to create the fragment
- the sense and antisense sequences of the fragment
- fragment chromatogram graphs, if that data is available
- an original unedited fragment sequence at the top of the pane where it can be edited and at bottom of the pane for reference only

Any editing changes in the Chromatogram Pane are immediately reflected in the Sequence Pane. Regions of the sequence may be copied and pasted to/from the Clipboard.



Navigating the Chromatogram Pane Using the Keyboard

Standard keyboard arrow keys can be used to navigate the chromatogram.

A way to define an exact caret position is to pause the caret over a residue for a moment allowing a popup label to identify its position.

To position the caret specifically, press the Set Caret Position box on the status bar. In the Set Caret Position dialog box that opens, enter the caret position and click OK.

Resizing the Chromatogram

To re-size the chromatogram, use the **Zoom In**  or **Zoom Out**  buttons or select **View > Zoom In** or **Zoom Out** or the corresponding commands on the shortcut menu. Chromatogram peak height can be adjusted with the vertical scroll lever on the left side of the Chromatogram Pane.

Fragment Window Operations

Editing a Sequence

Note: If the fragment you are editing in the Fragment Window is not included in any assembly, then you can edit that fragment freely. When you save your changes using the File/Save commands or by pressing CTRL-S, the fragment is not saved on a disk, but the fragment in the main fragments list in the current project is updated. To save your changes on disk, save your changes first in the Fragment Window, and then either save the current

project or export the fragment to disk. You can perform these operations in Project Explorer Window. *See page 478 for more information on how to save projects and export fragments.*

The fragment you are editing in Fragment Window can be included in an assembly or in several assemblies. In this case, the logic becomes a bit more complex:

- If you don't change the length of the fragment while editing, that is, you just replace the symbols, then the consensus for all contigs where this fragment has been included is recalculated after you save the fragment.

If you decide to insert or delete symbols, then the following warning message appears (Fig. 30.6):



Fig. 30. 6 Warning message regarding editing the fragment

If you press **Yes**, then you may edit the fragment freely. However, when you save your changes into the project, the program asks you again to confirm that you want to save the fragment and informs you that it will delete all assemblies that contain this fragment. Be careful, as deleting assemblies is cannot be undone—you may lose your contigs forever!

If you press **No**, then any further attempt to insert or delete nucleotide symbols will be ignored. No assemblies will be deleted after you save the fragment into the project.

You can edit the sequence in the Sequence or Chromatogram Panes in the same way you would edit text in a word processor with the following exceptions:

- In the Sequence Pane, only the direct strand can be edited. *The complementary strand is automatically updated.*
- In the Chromatogram Pane, only the top sequence can be edited. The original fragment sequence at the bottom is included for reference only.

The valid symbols you can enter are A, T, G, C, N and other acceptable ambiguous nucleotide designations specified by IUB codes (see Appendix C). Editing changes in either pane are reflected in the other pane immediately.

Sequence editing techniques are detailed in the following table:

Editing Operation	Action
Delete a nucleotide	Position the caret to the left of the symbol to be deleted (note caret position on the status bar); press DELETE. Residue is

Editing Operation	Action
	deleted and its position is "filled" with a gap. Position the caret to the right of the symbol to be deleted; press the BACKSPACE key. Residue is deleted and its position is filled with a gap.
Insert a symbol	Position the caret where the new symbol is to be inserted. Type a valid symbol (A, T, G, C, N or other accepted ambiguous code). The new symbol (colored) is inserted.
Replace a symbol	Select the normal symbol (or gap symbol). Type a valid new symbol (colored), which replaces the previous one. Several symbols can be replaced at one time: select them and enter the new symbols.
Paste a sequence from the clipboard	Position the caret where the symbols are to be inserted, or select the part of the sequence you want to be replaced. Press CTRL-V or Edit > Paste...
Cut a selection	Select the sequence region to be cut. Press CTRL-X or choose Edit > Cut . The selected symbols are copied to the clipboard and deleted from the sequence.

Table 30. 3 Sequence editing techniques

Cut, Copy and Paste options are also available on the shortcut menu opened from the Sequence Pane.

All editing operations are reflected in both the Sequence and Chromatogram Panes. Editing operations and results are *summarized* as follows:

Action	How to Perform	Sequence Pane Result	Chromatogram Pane Result
Delete	Select residues; press DELETE	(↓) replaces NTs; NTs (red) move below strand	(- - -) appear in upper sequence
Insert	Place caret; type new NTs	(↑) appears below new NTs; new NTs are green	New NTs are green; a break appears in the chromatogram

Action	How to Perform	Sequence Pan Result	Chromatogram Pane Result
Replace	Select NTs; type new NTs	New NTs (blue) appear in strand; replaced NTs move below strand	New NTs (green) appear in upper sequence; no break in chromatogram

Table 30. 4 Editing operations and results

Calling Secondary Peaks

At times, it seems desirable to review secondary peaks on a chromatogram and even change sequence residues based on unreliable chromatogram values. ContigExpress searches for secondary peaks by looking for lower peaks that are at least as tall as some percentage of the highest peak at a base call position. For details on calling secondary peaks, refer to Chapter 31.

Showing Deleted Peaks


By default, peaks for deleted bases are shown in the Chromatogram Pane of the Fragment Window. Peaks for deleted bases can be removed from the chromatogram by using choosing **View > Show Deleted Peaks** from the menu or by pressing the Show Deleted Peaks toggle button on the toolbar. The advantage of removing deleted peaks is that the fragment sequence can then be translated across a deletion region in the new frame resulting from the deletion. Otherwise, if deleted base peaks are showing, translations proceed without considering the deletions. For more information regarding Showing Deleted Peaks, see Chapter 17.

Showing Traces

By default, all traces are shown in the Chromatogram Pane of the Fragment Window. Traces for each base can be toggled off and on independently in the Chromatogram Pane. Choose **View > Show Trace > Trace<base>** from the menu or press the **Show Trace** button for the particular base on the toolbar to hide (or show) the trace for that base.

Window Viewing Options

To modify default viewing options for the Fragment Viewer, click on the **Viewing Options**

button () on the Viewer Toolbar or select **View > View Options**. This opens the Fragment Viewing Options dialog box (Fig. 30.7):

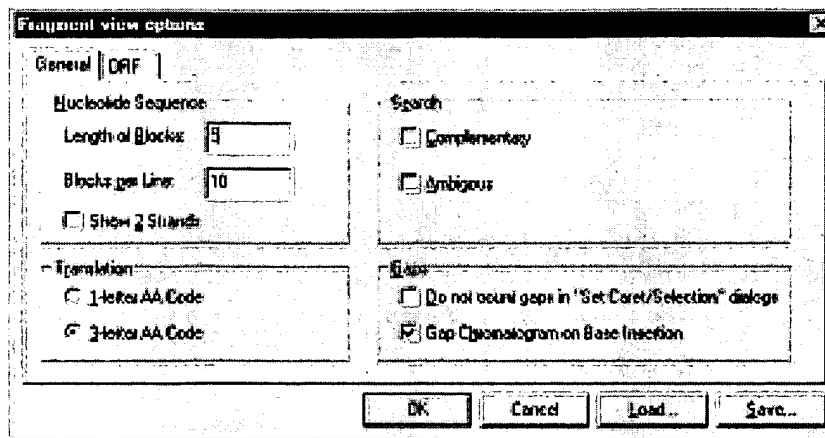


Fig. 30. 7 General tab on the Fragment Viewing Options dialog box

On the **General** tab, you can review or change the default settings in the dialog box:

- **Nucleotide Sequence** length of a single line of a sequence, the number of blocks per line, single or double stranded display (Sequence Pane)
- **Search:** default settings for the Find dialog box
- **Translation:** three- or one-letter protein codes displayed in the sequence translation.
- **Gaps:** default settings for the Do Not Count Gaps checkbox in the Set Caret Position and Set Selection dialog boxes. The second checkbox, Gap Chromatogram on Base Insertion, controls how new bases are inserted into an editable sequence. You may either choose to gap a chromatogram graph on base insertion (check the box), or insert a new base without changing a graph (leave the box unchecked).

On the **ORF** tab, you can set the parameters for ORFs display in the Fragment window (Fig. 30.8):

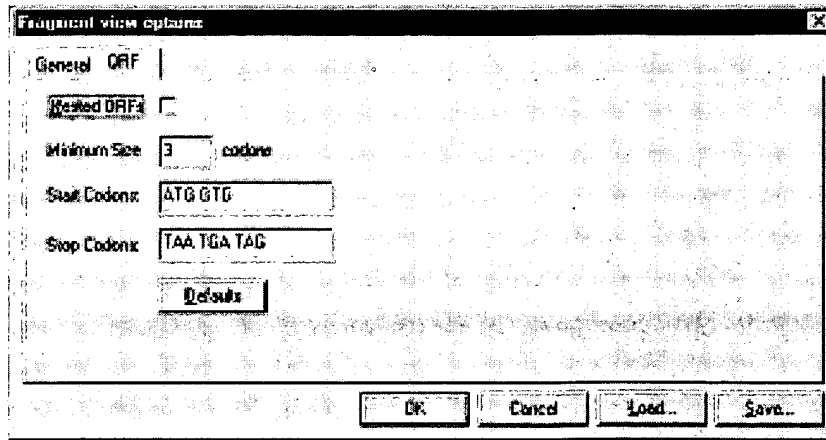


Fig. 30. 8 ORF tab on the Fragment Viewing Options dialog box

- **Nested ORFs:** Check the box to display ORFs that occur within the main ORF.
- **Minimum Size:** Specify the minimum ORF size in codons that you want to display.
- **Start Codons:** Define acceptable start codons.
- **Stop Codons:** Define acceptable stop codons.
- **Defaults button:** Restores ORF dialog box default values.

Contig Window

Launching a Contig Window

To open a Contig Window, double-click on a contig in CE Project Explorer, or select **Open** on a shortcut menu opened from a contig in Project Explorer. Contig Window is one of the modes of the ContigExpress workspace described in Chapter 2.

Features of a Contig Window

The ContigExpress Contig Window (Fig. 30.9) consists of a menu bar, two toolbars and is divided into three panes: a Text Pane, a Graphics Pane and a Contig Alignment Pane.

A shortcut menu associated with specific folders or objects can be opened with a right click while the cursor is on the object. Toolbar buttons are displayed in this section as needed. All of the toolbar buttons are described in Chapter 4.

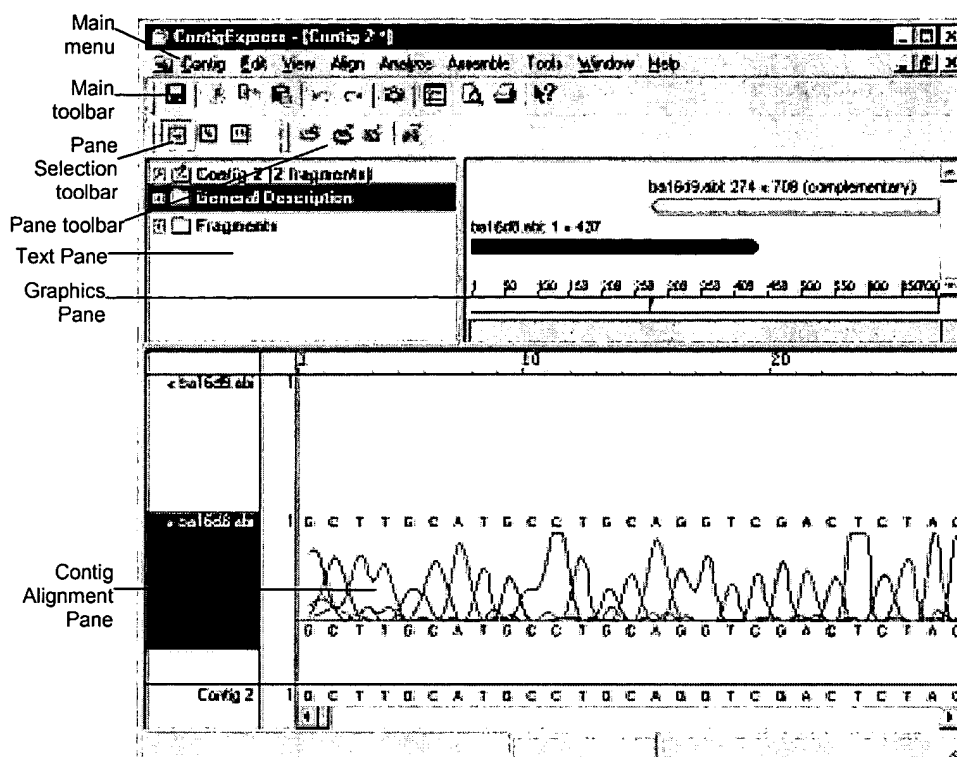



Fig. 30. 9 ContigExpress Contig window

As in the other Vector NTI Suite applications, to apply a command from the Main Menu in a given pane, that pane must be active. The active pane can be toggled with the Switch Pane buttons () on the Pane Selection Toolbar or by clicking in the pane you want to activate. You can also activate the panes with the F6 and SHIFT-F6 keyboard buttons.

Text Pane

The Contig Window Text Pane contains a thorough description of the contig displayed in the window.

Text Pane manipulations are outlined in the previous chapter and are also summarized in chapter 3.

The Text Pane data is divided into a hierarchy of folders and subfolders. A Contig Text Pane can contain the following folders:


Folder	Contents
General description	Name, creation date, length, etc.
Comment	Arbitrary text of any length associated with the contig
Fragments	List of fragments contained in the contig

*Table 30. 5 Contig Text Pane folders***Graphics Pane**

The Contig Window Graphics Pane (Fig. 30.10) contains horizontal arrows representing the relative positions of the fragments forming the contig. The arrowheads indicate whether the respective fragment is in the direct or complementary strand, with the names of the fragments displayed above the fragment lines. When either of the Graphics or Alignment Panes is active, one of the fragments is always highlighted with another color (usually dark blue). This highlight is coordinated with highlights in the Sequence Pane to allow you to locate the same fragment in all panes.

When the Contig Window first opens, all data in the Graph pane is in the Fit to screen mode. In this mode all the graphical data for the complete contig is always shown in the pane no matter how you resize the pane.

If you want to examine a part of the graph pane, you can resize the pane using the Zoom and Fit to Screen buttons. Resizing of the pane does not affect the current scale of the graph. For more information on using the Zoom buttons, refer to chapter 3.

By default, zooming is done horizontally. If you want to zoom vertically, press CTRL + press the **Zoom In** button (.

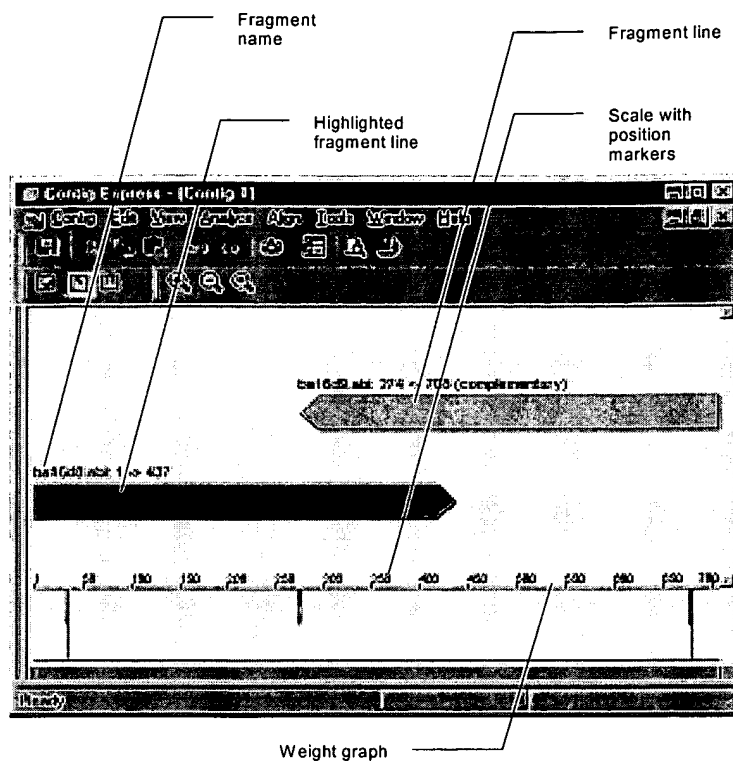


Fig. 30. 10 Contig Window Graphics Pane

Below the fragment arrows is a scale or Weight Graph, with position markers for this contig. The Weight Graph displays the quality of the assembly. You may note on the graph small green vertical lines dropping below the graph. If you position your cursor at one of those sites and press the **Zoom In** button many times (until it is disabled), you can see the exact nucleotide placement of each line in the graph. You will also note the negative peak in the Weight graph line, indicating a position where the assembly is poor (Fig. 30.11). Refer to the same position(s) in the chromatogram and you will see poorly resolved peaks.

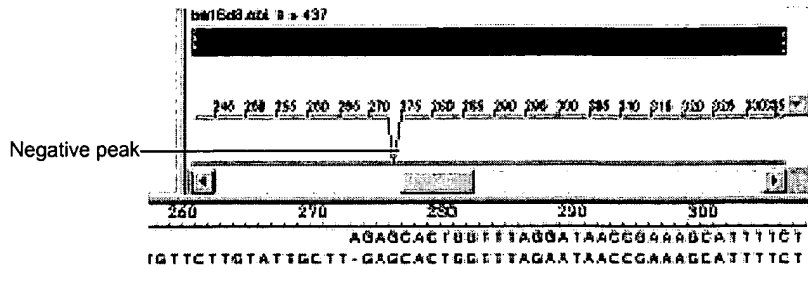


Fig. 30.11 Negative peak in the Weight graph line

In the Weight Graph, each combination of two possible sequence symbols is assigned a certain weight. For each position in the contig, the resulting weight is calculated from all symbols at this position in the fragments available for this position and from the consensus symbol at this position. The resulting weight is plotted as a point in the Weight Graph. Dips in the line isolate places where the assembly is poor or nucleotides are mismatched.

By default, the highest weights are given to combinations of equal symbols, for example, "AA" or "CC". The weights can be changed in the Viewing Options dialog of the Contig Window.

ORFs can be shown for the fragments in the Graphics Pane. Choose **View > Show ORFs** from the menu or press the **Show ORFs** button to add the ORFs display to the Graphics Pane.

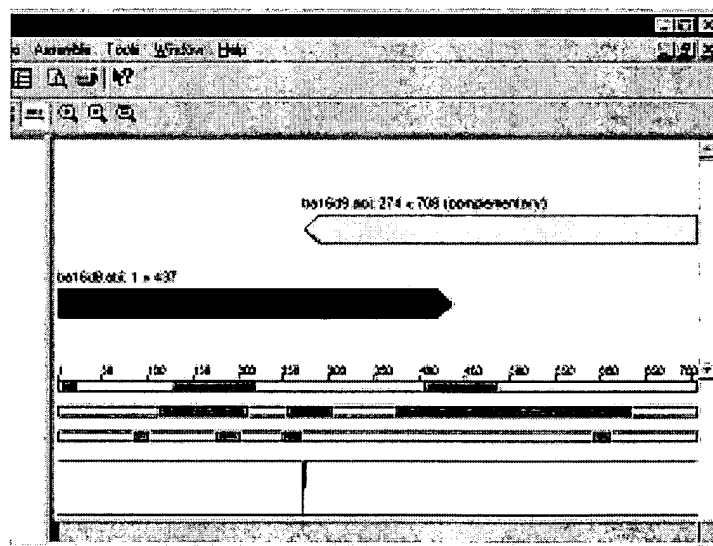


Fig. 30.12 Green bars depict ORFs in the fragments

Green bars in the ORFs display area depict ORFs in the fragments (Fig. 30.12). Clicking on a green bar highlights that ORF in both the Graphics and Alignment Panes.

Open a Fragment In Another Window

From the Graphics Pane, double-click on a fragment line to open the fragment in a separate Fragment Window.

Camera Specifics

While you can use the Camera feature to copy pane contents, only the currently visible portion is copied to the clipboard or file. In this pane the Camera cannot copy entire selections.

The data is copied to the clipboard or file in the Windows metafile format (WMF).

For more information on the Camera copy feature, refer to chapter 3.

Alignment Pane

The Contig Alignment Pane (Fig. 30.13) displays the nucleotide sequences of the fragments that form the contig with overlapping regions aligned appropriately and displayed relative to their positions in the contig. You can edit the sequences here and see how your actions are reflected in the contig. The respective chromatograms for the sequences, also aligned appropriately, can also be displayed on command.

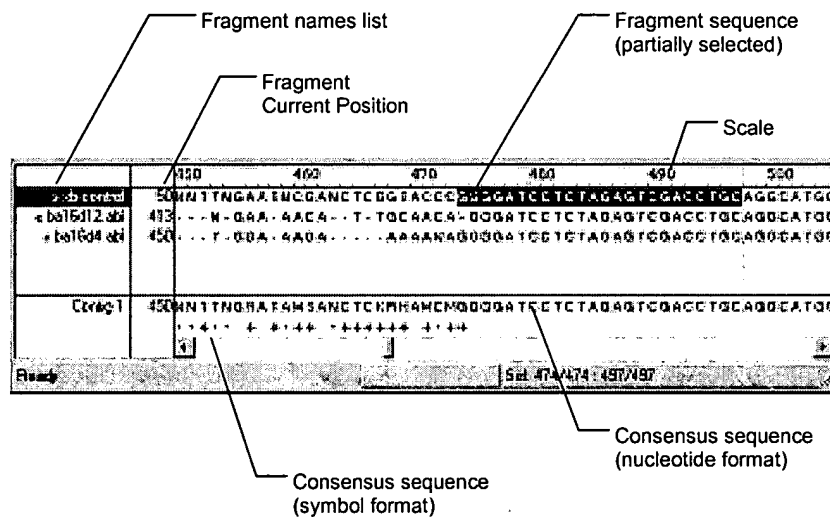


Fig. 30. 13 Contig Alignment Pane

The Contig Alignment Pane has the following main elements:

- The **fragments names list** (at the left) contains all fragments that are used in this contig.
- The **fragment current position** (below the scale current position) is the relative position of the left end of the fragment (counted from the fragment start) that is currently shown.
- The **scale** (above the chromatogram alignments) gives a reference nucleotide position for all fragments and the consensus shown in the alignment.
- The **consensus sequence (nucleotide format)** shows the nucleotide consensus for the assembly.
- The **consensus sequence (symbol format)** shows the symbol consensus for the assembly. In the symbol consensus, blank spaces indicate identical residues, dots (•) indicate positions containing Ns and plus symbols (+) indicate ambiguity and/or gaps.
- The **fragment** itself includes its *editable sequence* and (if its chromatogram is switched on) a chromatogram and original sequence (which does not change even if you edit the fragment).

When either of the Graphics or Alignment Panes is active, one of the fragments is always highlighted (its name in the fragment names list at the far left) and selected concurrently in other pane. To highlight another fragment, simply click on it.

Finding Sequence Fragments

A specific sequence fragment can be found in the Alignment Pane using the Find feature. Click on a fragment name in the Alignment pane list to select it. Launch the Find sequence dialog box either by choosing **Edit > Find** from the menu or by pressing the **Find** button on the toolbar. When Find dialog box (Fig. 30.14) appears, type in the sequence you want to find.

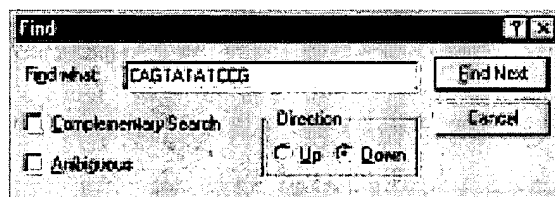




Fig. 30. 14 Find dialog box

Press the **Find Next** button. If the sequence is found, the Alignment Pane displays the region where the sequence has been found and highlights the area.


Find Next/Previous Ambiguous Symbol

To search a contig's consensus sequence for ambiguous symbols (like "N" or "R"), click on the **Find Previous**  or **Next Ambiguous**  buttons or select **View > Find Previous** or **Next Ambiguous**.

If you start this search with a sequence selected, the search proceeds from the selection in the specified direction. If there is no selection, the search starts from the start (Find Next) or the end (Find Previous) of the contig.

If an ambiguous symbol is found, its position is selected and displayed. If there are no more ambiguous symbols in the specified direction, you are informed as such.

Switch the Fragment's Chromatogram On/Off

To alternate the display of the chromatogram with the original sequence of the highlighted fragment, click on the **Show Chromatogram** button  on the Pane Toolbar or select **Show Chromatogram** on the shortcut menu. You can also select **View > Hide/Show All Chromatograms** or the corresponding command from the shortcut menu. *If the fragment does not have a chromatogram, you will be informed with an intercept message.*

Editing Contig Fragments or Consensus

You can use the Alignment Pane to edit the fragments that comprise the contig, change their positions or orientation or edit the consensus itself. All editing changes in the fragments cause an immediate recalculation and redisplay of the contig's consensus sequence. Editing changes in the contig consensus are reflected immediately in the fragment sequences that comprise the contig.

Although the basic sequence editing techniques are the same as in the Sequence Pane of the Fragment Window, there are some editing limitations and specifics because the fragments are incorporated in a contig. Since the fragments that are incorporated in a contig can also be present in other contigs, such edit operations are allowed only after you confirm that you are aware that any other assemblies containing the fragment will be dismissed:

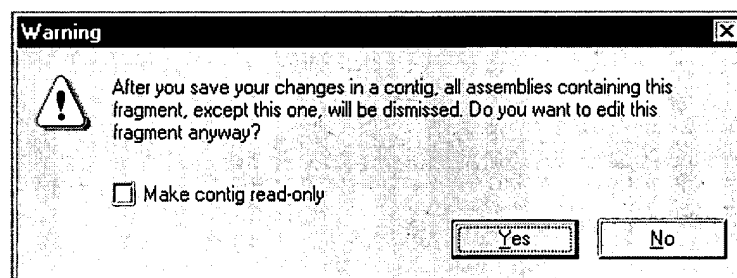


Fig. 30. 15 Warning message regarding editing the contig

Note: This warning only appears the first time you attempt editing changes (Fig. 30.15). If you select **Yes**, you will be allowed to continue making more changes without warning.

Click **Yes** to accept the changes. If you check the **Make contig read-only** box, the Yes option is disabled. If you select **No**, you are returned to the Contig Window.

If your changes are base deletions, the nucleotides are deleted from the chromatogram and the gap is closed, unlike deletions in the Fragment Window, which are indicated by dashes in the Chromatogram Pane. The consensus is changed in that bases to the right of the deleted region now move to the left, filling the gap and a vertical marker appears in the sequence at the position of the deletion.

Although there is now no indication in Contig Window that these changes were made, if you were to save the edited contig, then re-open it and open the edited fragment in the Fragment Window, the editing changes would be reflected in the sequence.

Undo/Redo functions can be used to revert or repeat the changes you make. Click the **Undo** button until it becomes disabled and the fragment and the consensus return to their original state. You can also select **Contig or Fragment > Revert to Saved**, *depending upon the open window*.

To help you track modifications in the contig, new symbols are displayed with a different color. If the chromatogram for the fragment is displayed, you can also compare the edited sequence with the original sequence below the chromatogram.

Translating the Consensus

The consensus sequence translation can be displayed in the Alignment Pane in any one or all of the three direct frames. Display (or hide) the consensus translation in a particular frame by choosing the corresponding **View > Consensus Translation > Frame** command from the menu or by pressing its **Consensus Translation - <#> Frame** button counterpart.

Operations in Either the Contig Pane or Alignment Pane of Contig Window

The following operations can be performed similarly in either the Contig or Alignment Panes:

Highlight a fragment

To highlight a fragment, click on a fragment “arrow” in the Contig Pane or on a fragment name in the Alignment Pane. The same fragment is concurrently selected in other panes of the window.

Moving a Fragment

This action changes the position of a fragment in the contig. This operation is possible only when there are adjacent gaps in the direction of its movement or if the fragment relocation does not create any gaps in the contig.





Note: If the Move Fragment buttons and menu options are disabled when you try to edit, the contig window may be in 'Read-Only' mode (indicated on the title bar). When you started the editing, a message box appeared informing you that editing the contig would cause all assemblies to be dismissed.

Three Methods to Move a (Highlighted) Fragment in the Contig Pane:

1. Press CTRL + DRAG the highlighted fragment block to the new location. You will see a transparent copy of the fragment following the movement of your cursor. The new position of the fragment being dragged is shown in both a pop-up label and the workspace Status Bar.


Since fragment block moving is not allowed to introduce gaps into the contig, your ability to drag the block will be appropriately limited. When you release the left mouse button, the fragment "drops" into the new position, the contig consensus is recalculated and all panes of the Contig Window are updated. To cancel fragment moving, press **Esc** while dragging the fragment. *Fragment moving is cancelled if you switch to another window before dropping the fragment.*



2. Select **View > Move Fragment ...** or the corresponding command on the shortcut menu. In the dialog box that opens, enter the fragment position change in base pairs and select the move direction (right or left). Click **OK** to move the fragment to the new position. The contig consensus is recalculated; all panes of the Contig Window are updated.

3. Click one of the **Move Fragment** buttons (, , , ). One click moves the highlighted fragment one position in the respective direction. When movement in any direction is no longer possible, the respective button becomes disabled.

Two Methods to Move a (Highlighted) Sequence or Block in the Alignment Pane.


Note: The following editing changes may be easier to monitor if you make them in the

Chromatogram View. Click the **Show All Chromatograms** button () , select **View > Show All Chromatograms** or the comparable command on the shortcut menu.

1. To delete bases, select them and press **DELETE**. The consensus sequence at the pane lower edge now reflects the discrepancy, although the contig does not give any clear indication of the deletion.
2. Place the cursor between two nucleotides and press the space bar, creating a gap (shown by dashes). Now select bases next to the gap, enabling the Move Fragment button(s) (, ). Press the button that allows you to shift the block into the gap (or select **View > Move Selected Fragment**).

Reverse Complement a Fragment


To reverse a fragment in a contig, highlight the fragment, click the **Reverse Complement**

Fragment button () , select **View > Reverse Complement Fragment** or the corresponding command from the shortcut menu. The fragment will be reverse

complemented, the contig consensus are recalculated and all panes of the Contig Window are updated.

Window Viewing Options

To review or modify default viewing options for Contig Window, click on the **Viewing**

Options button (). This opens the Contig Viewing Options dialog box (Fig. 30.16):

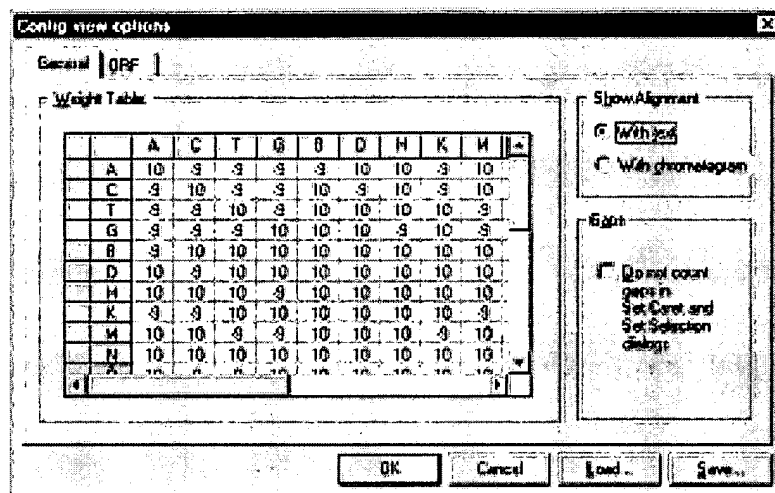


Fig. 30. 16 General tab of the Contig Viewing Options dialog box

The Weight Table on the **General** tab allows you change the nucleotide symbol weight coefficients used in calculation of the Weight Graph data in the Graphics Pane. To change a coefficient for any nucleotide symbol pair, click in the table cell that is formed by the intersection of the nucleotide pair and enter a new value. The table has diagonal symmetry because the order of the symbols in the pair is irrelevant; the changes in one half of the table are instantly mirrored in the other half.

- **Show Alignment As** selects whether to show chromatograms in the Alignment Pane
- **Gaps** sets a default setting for the Do not count gaps checkbox in the Set Selection dialog box

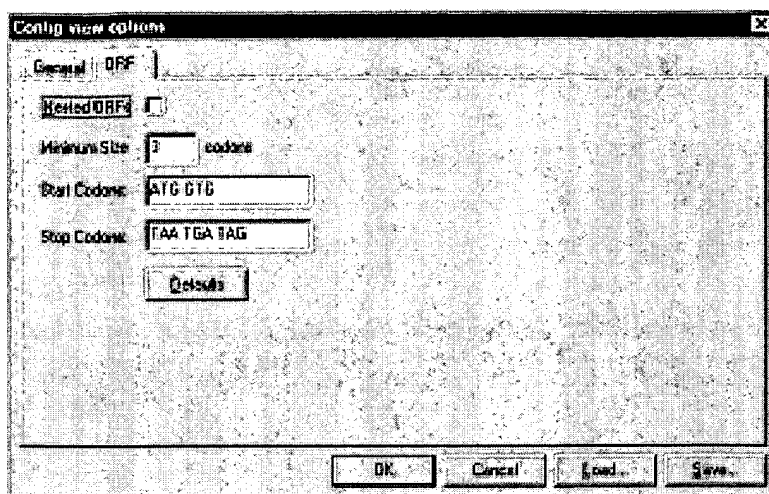


Fig. 30. 17 ORF tab of the Contig Viewing Options dialog box

The **ORFs** tab (Fig. 30.17) allows you to define the parameters for the ORFs search. Start and stop codons can be defined here, as well as the minimum codon size for the search and whether or not nested ORFs will be displayed. Pressing the **Defaults** button restores ORF parameters to their default values.

Copy and Camera Features

The Copy feature is the same as in other panes except that only the selected part of a highlighted fragment is copied. The Camera feature for this pane works in a slightly different manner than it does in other windows.

When you select this action, you will see the Camera dialog box (Fig. 30.18):

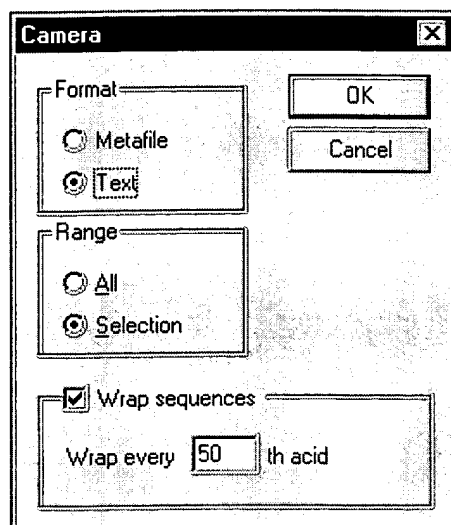


Fig. 30. 18 Camera dialog box

In the Metafile format, only the currently visible part of the Alignment Pane can be copied to the clipboard. The data is copied in the Windows metafile format (WMF).

In the Text format, you can select whether you want to copy a current selection or all data available in the contig (it is not limited to the visible part). You can also set wrapping of the copied sequences at every nth position. The data is copied in the plain text and Rich Text (RTF) formats.

Chapter 31 ContigExpress: Group Operations on Fragments

Introduction

This chapter consists of operations performed on fragments to optimize contig assembly. Operations covered are fragment and vector contamination trimming and calling secondary peaks.

Fragment Trimming

Fragment trimming is an operation performed on chromatogram fragments to optimize sequencing results and contig assembly. Trimming is performed on fragment ends to remove unresolved or poor quality nucleotides based on chromatogram results. It is also used to remove bases identical to restriction sites or other sequences from a vector. An entire chapter is devoted to this operation because of its complexity.

All fragment trimming is a multi-step process in Vector NTI Suite. Steps in the process are summarized as follows:

1. Open the Fragment Ends (or Vector Contamination) Trimmer dialog box where trimming can be performed on a group of fragments.
2. Define the trimmer settings
3. Calculate the trimming using the parameters you defined. A preview is provided so you can see where the fragments will be trimmed.
4. Select or unselect all of the ends to be trimmed
5. Complete the trimming process by closing the dialog box with the **OK** button.

Fragment Ends Trimmer dialog box

In the ContigExpress Project Explorer, select the fragments to be examined and prepared for trimming. Select **Edit > Trim Selected Fragment Ends**, opening the Fragment Ends Trimmer dialog box (Fig. 31.1).

When first opened, the dialog box displays the default settings for the type of trim used. While the dialog box is open, you can change the trimming settings and recalculate the trims as many times as you wish.

The dialog box contains a scrollable list of Fragment Mini-windows for all the fragments you selected for trimming in Project Explorer. To facilitate viewing the trim locations, this dialog can be resized like any other window using the keyboard or mouse.

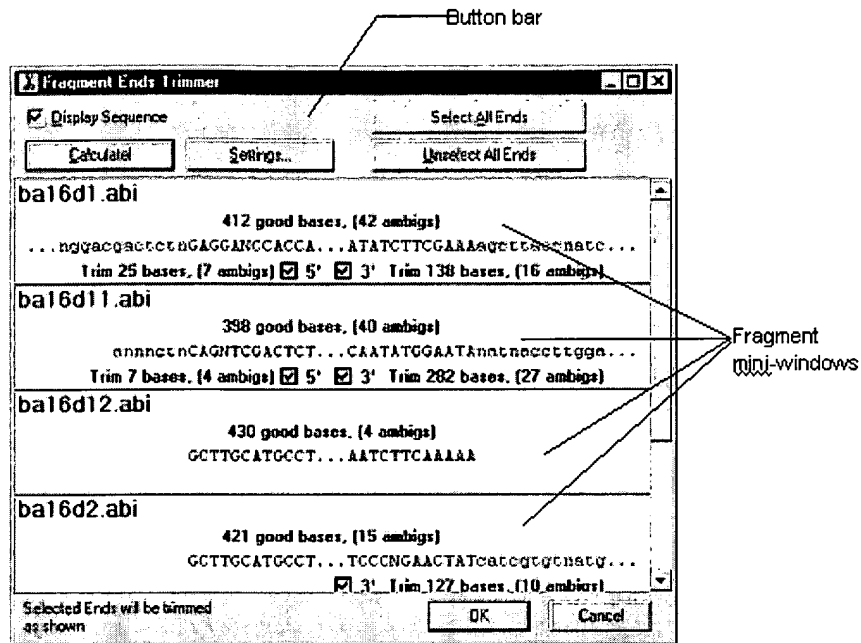


Fig. 31. 1 Fragment Ends Trimmer dialog box

The Fragment Trimmer dialog box buttons are described in the steps below. It is suggested that you follow them as numbered.

The fragments you selected in the Project Explorer are listed in “mini-windows” in this dialog box. Each mini-window shows one fragment with information on how the current trim will affect it. In this window, you can select those trim locations to be used in the final trim for the fragment in the project.

When the fragments are displayed as sequences (when the Display Sequence box is checked), the Fragment Mini-window (Fig. 31.2) has the following elements. (Some of the elements do not appear until after the calculation step):

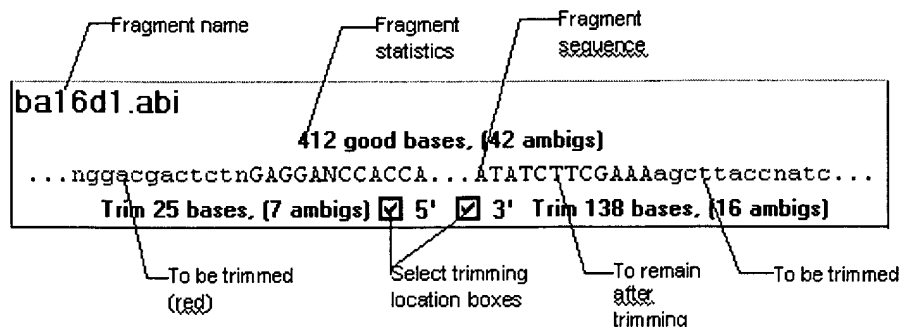


Fig. 31. 2 Fragment Mini-window when fragments are displayed as sequences

- **Fragment name** is the name of the fragment in the ContigExpress Project;
- **Fragment statistics** show the number of good (non-ambiguous) bases and the number of ambiguous bases in the fragment;
- **Fragment sequence** is the nucleotide sequence of the fragment; the ends that will be trimmed are in lowercase and selected in the red color, all the rest will remain intact after trimming;
- **Trim statistics** shows the amount of bases that will be trimmed off the fragment from each end and the amount of ambiguous bases among them;
- **Select trimming location boxes** allow you to select the ends that will be trimmed.

Due to window size constraints, usually only the most important parts of the sequence, the places around the trim locations, are displayed. Omitted parts of the sequence are replaced with ellipses (...). To view the entire fragment in a Fragment Display Window (in Read-Only mode), double click anywhere in a Mini-Window

To display fragments in a graphical form, with a bar differentiating (in different colors) the parts for trimming from the remaining sequence part as seen below, uncheck the Display Sequence box. The result is shown below (Fig. 31.3):

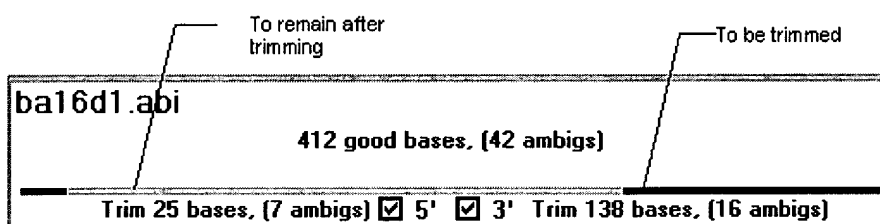


Fig. 31. 3 Fragment Mini-window when fragments are displayed in graphical form

Follow the trimming steps in the numbered order:

1. Set fragment display options

To establish the fragment display format, check or uncheck the Fragment Display box or press alt-D.

2. Define trimmer settings

Click on the **Settings** button or press alt-S, opening the Trimming Settings dialog box. The contents of this dialog box may vary according to the type of the Fragment Trimmer dialog box. See the section on Vector Contamination Trimming for more details.

Fragment Ends Trimming Settings dialog box

The settings defined in this Trimmer Settings dialog box (Fig. 31.4) determine how contig fragments' ends are trimmed. When the dialog box is opened, its fields are filled with the current settings. To load previously saved parameters, press the **Load** button.

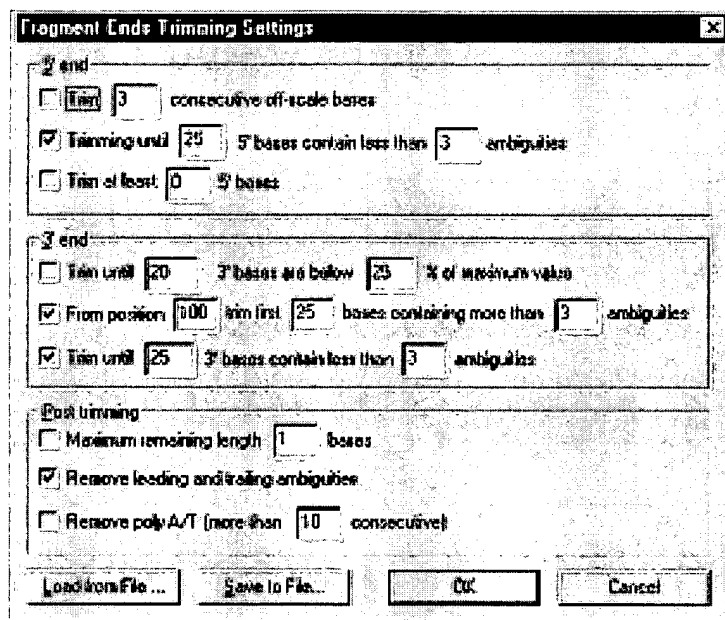


Fig. 31. 4 Fragment Ends Trimming Settings dialog box

5' end:

- **Trim off-scale bases** removes the defined # of consecutive bases that are below acceptable criteria
- **Trimming until __ 5' bases ...** can be based upon chromatogram quality
- **Trim at least ...** is an arbitrary setting that may be based upon the fact that your primers have "tails"

3' end:

- **Trim until __ 3' bases ...** removes bases whose peaks do not meet the % value you define
- **From position ... trim first...** removes these bases if you can see that quality well into the sequence is not good
- **Trim at least...** removes bases that begin to be poorly resolved at the 3' end

Post trimming

- **Define maximum ...** set the length of the fragment that must be left after trimming
- **Leading and trailing ...** removes poorly resolves nucleotides that may be left after trimming
- **Remove poly A/T...** removes these nucleotides that may be present if the sequence was flipped, producing a poly-T 5' end.

Press **Save** to save these settings as a file to be used in the future. Press **OK** to apply the new settings in the current trimming exercise and return you to the Fragment Ends Trimmer dialog box. This also saves these settings as default settings for all future trim calculations. To cancel changes, press the **Cancel** button.

If you calculated the trims before redefining these settings, you must recalculate after closing the Settings dialog box to view trimmings according to the new settings.

3. Calculate the trim

Calculate! shows the potential trimming under the parameters you set in the Trimmings Settings dialog box. To calculate the trim for all fragments using the current settings, press the **Calculate!** button or press alt-C. Since trim calculation can be a lengthy process, a progress dialog box appears with a progress bar (Fig. 31.5). *Press the **Cancel** button or the ESC key to stop the process.*

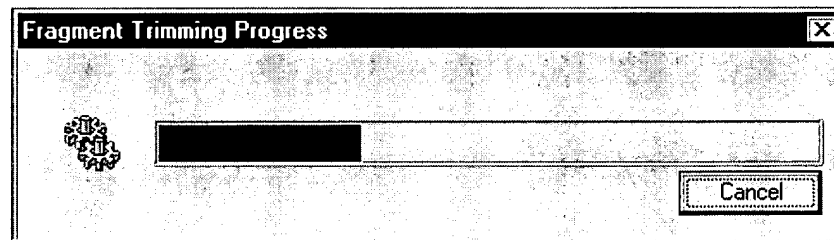


Fig. 31. 5 Progress dialog box with progress bar

If the trim calculation process is allowed to complete, all fragments are updated with newly calculated trim locations, displayed in red, lower-case nucleotides in the mini-windows.

4. Select trimming locations

Press the **Select All** or press Alt-A to select all of the trim locations displayed in the mini-windows for the final fragment trimming. click the **Select All** or **Unselect All** buttons or use keystrokes Alt-A or Alt-U respectively. Note that all of the checkboxes for the ends are checked as in this example: ☒ 5'

For manually selecting trim locations on individual molecules for final fragment trimming, press the **Unselect All** buttons in the Fragment Ends Trimmer dialog box. This unchecks the 5' and 3' boxes for each fragment in the mini-windows. In the mini-window(s) in which you wish to select the trim locations, click on the appropriate fragment check boxes, ☒ 5', ☐ 3'.

When a location is selected, the respective part of the fragment will be trimmed off when the trimming is completed.

5. Confirm the proposed trimming operation

To open a Fragment Window for any fragment in a mini-window so that you can review and compare prospective fragment trimming with the corresponding chromatogram, double-click anywhere in the mini-window. *The Fragment Window that opens is in read-only mode.*

As this window is created, the fragment that will remain after trimming is selected in the fragment sequence, both in the Sequence Pane and in the top sequence of the Chromatogram Pane. *The fragment opened this way is always opened in read-only mode.*

If the Fragment Window for the fragment already exists, the selection is made inside it and it is brought to the foreground.

6. Complete the trimming operation

Once parameters and trimming conditions are established, click **OK** to close the dialog box and proceed with the fragment trimming. To close the dialog box without changing the fragments in the project, select the **Cancel** button or press the ESC key.

Vector Contamination Trimming Settings Dialog Box

The purpose of the Vector Contamination Trimming feature is to trim from sequenced fragments any residues from your frequently used cloning vectors that may have been amplified in the sequencing process.

In the Vector NTI Database Explorer, highlight the cloning vector in the List Pane, or open a vector in a Molecule Display window and select a sequence such as a frequently-used polylinker site. Select **Tools > Send to > Polylinker to ContigExpress**.

In the Sequence Data dialog box that opens, indicate whether you are sending the entire sequence or the selection and the direct or complementary strand. Click **OK**, opening the Choose Polylinker file name dialog box. In naming the sequence, make sure you retain the

.seq extension because this is the only way ContigExpress recognizes this sequence for the trimming operation. Click **Save** to save the sequence in the VecContData folder.

Notes about sending sequences for vector trimming:

- From Database Explorer, you can send only one vector at a time.
- If you have been sequencing off of the complementary strand, be sure and indicate this, as only one strand is forwarded to ContigExpress.
- In naming the sequence, make sure you retain the .seq extension because this is the only way ContigExpress recognizes the sequence for the trimming operation.

Now in the Project Explorer in ContigExpress, select the sequenced fragments to be trimmed and choose **Edit > Trim Selected Fragments for Vector Contamination**. This opens the Fragment Vector Contamination Trimmer dialog box, (not shown but similar to the Ends Trimmer dialog box).

In this dialog box, press the **Settings** button to open the Fragment Vector Contamination Trimmer Settings dialog box (Fig. 31.6). The vectors or polylinker sites you selected in the Database Explorer should be listed in the List Pane at the left. The Edit Pane where you can edit the vector sequence is on the right.

Tip: If when you open this dialog box, the Polylinker List Pane is empty, there can be two reasons/solutions:

1. Return to the Project Explorer, and select your vector(s) or polylinker(s) as described above.
2. You may have selected the vector/polylinker correctly, but you lost the .seq extension in naming them when they were saved in the VecContData folder.

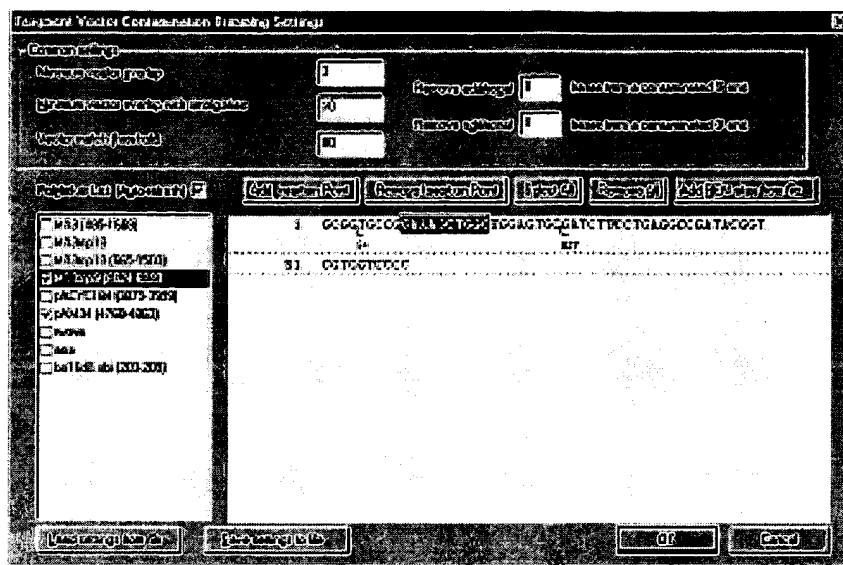


Fig. 31. 6 Fragment Vector Contamination Trimmer Settings dialog box

The Common Settings in this dialog box are described as follows:

- **Minimum vector overlap** is the minimum number of bases in the fragment that overlap with those on the clone. *This setting must be 5 or greater.*
- **Minimum vector overlap with ambiguities** includes poorly resolved residues
- **Vector match threshold** is the % required to match
- **Remove additional ... from 5'/3' end...** refers to the additional bases to be removed

The polylinker list in the Polylinker List Pane on the left is updated every time you open this dialog box. Check the Auto-refresh box to refresh the list every time you switch to ContigExpress from another application where the Vector Contamination Settings dialog box is open.

By default, a new polylinker is not selected for trimming and has no insertion points.

Editing a Polylinker

To select a polylinker for the trimming process, check the check box to the left of its name and highlight it by clicking on it. The Polylinker Edit Pane to the right of the list shows the contents of the selected polylinker. You can use this window for simple editing of the polylinker sequence and for managing the insertion points of the polylinker. Editing commands appear on the buttons; some are also available on a shortcut menu opened from the Edit Pane.

Insertion points can be added to the polylinker using two methods:

1. Manual adding: position the caret where you want to add the insertion point; press the INSERT key or click the **Add Insertion Point** button.
2. Import a group of insertion points using a file with an enzyme database (in Rebase format). Click the **Add REN sites** button or select **Add REN Sites...** from the shortcut menu. In the standard Open dialog box, you can select a Rebase file to be applied to the polylinker or simply the Enzlist.dat in the VecContData folder. Select **OK**, and the enzyme file is imported. The insertion points with the enzyme names are imported to the polylinker. Click on the enzyme name to select it as an insertion point.

The following options control the points:

- **Remove Insertion Point** deletes a selected point. *You can also press the DELETE key.*
- **Remove All Points** (on the shortcut menu) remove all insertion points from a polylinker
- **Select All** selects all insertion points. You can also just click on it. (Selected points are blue; an unselected point is gray.) *A corresponding option is also on the shortcut menu.* To be included in the trimming process, a point must be selected.
- **Remove Selected** deletes selected points. A corresponding option is also on the shortcut menu.

To toggle the selection status of an insertion point, click it with the mouse.

Click on the **OK** button to return to the Trimmings dialog box.

Executing the Vector Contamination Trim Changes

To complete the trimming process, follow the steps beginning with number 5 on page 511.

After all fragments are processed, the Fragment Trimmer dialog is closed and the Project Explorer contents are updated to reflect the changes.

Calling Secondary Peaks

ContigExpress can search for secondary peaks in a chromatogram by looking for lower peaks that are as tall as a percentage you define of the highest peak at a base call position (current threshold for secondary base calling). For example, a threshold of 70% would display secondary bases with heights 70% of the taller peak. This is specifically used for resolving the occurrence of double peaks in a chromatogram.

To initiate this process, in the ContigExpress Project Explorer window select fragments, then select **Edit > Call Secondary Peaks**. This opens the Call Secondary Peaks dialog box (Fig. 31.7) where you can review potential replacement bases determined by the search for secondary peaks for the group of fragments you selected.

To facilitate viewing the fragment sequences, this dialog can be resized like any other window by using the keyboard or mouse. The panes inside the dialog may be resized by moving the split bars.

The dialog box opens with the default settings for this operation which you can change as you wish. The dialog box has two panes: List Pane on the left with the fragments you selected in the Project Explorer and the Sequence Pane on the right displaying the sequence of the currently selected fragment in that list.

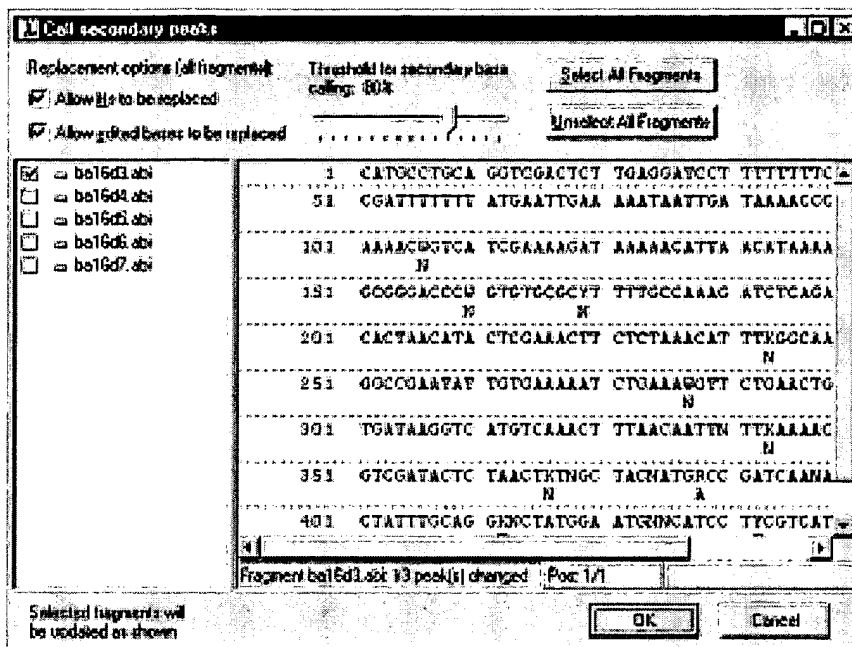


Fig. 31. 7 Call Secondary Peaks dialog box

The Call Secondary Peaks dialog box buttons are summarized as follows:

- **Allow Ns to be replaced** allows replacing the N nucleotide symbols.
- **Allow edited bases to be replaced** allows replacing of previously edited bases.

If either of the above settings are checked, the selected sequence is immediately regenerated to display a preview of the base that would be changed in the sequence.

- **Threshold for secondary base calling** defines the percentage of the height of the secondary peak in relation to the first for calling the secondary base
- **Select all fragments** indicates fragments to be saved into project after pressing **OK**
- **Unselect all fragments** indicates fragments are not to be saved in the project

List Pane

The List Pane shows the list of fragments you selected in Project Explorer for sequence editing. Select any fragment in the list to see a preview of the changes in the Sequence Pane. If you are satisfied with the results, check the box next to the fragment name to allow the displayed changes for this fragment to be saved in the project.

Sequence Pane

This pane displays the preview of changes that will be applied to the sequence using the current call peaks settings. The peaks that will be changed are shown in blue color. The original peaks are shown right under the changed peaks so you can easily track changes.

The user operations in the Sequence Pane are basically the same as in the Fragment Window Sequence Pane except that you cannot edit the sequence manually.

Status Bar

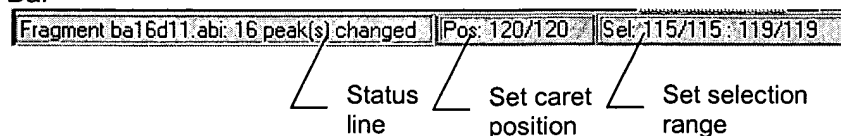


Fig. 31. 8 Status bar of the Call Secondary Peaks dialog box

The status bar of the Call Secondary Peaks dialog box (Fig. 31.8) shows the name of currently selected fragment in List Pane, and the number of peaks that have changed in its sequence. The Set Caret Position and the Set Selection Range boxes shows the caret position and a currently selected range of residues in the Sequence Pane. Pressing either of these buttons opens a dialog box where you can set a caret position or range directly.

After you have previewed the changes, check the check box to maintain the changes while you review other fragments. To select other fragments to be changed, click on their fragment IDs in the List Pane.

Executing Calling Peaks Changes

The changes are applied for those fragments checked in List Pane only. To save your changes in the project, press the **OK** button. If any of the affected fragments are included in assemblies, you will be given the option of dismissing the respective assemblies or canceling the changes for that particular fragment.

Chapter 32 BLAST Search and BLAST Viewer

Introduction

BLAST (Basic Local Alignment Search Tool) searches explore, using a DNA or protein query sequence, available sequence databases for sequence similarity. BLAST programs have been designed for speed, with a minimal sacrifice of sensitivity to distant sequence relationships. BLAST scores have a well-defined statistical interpretation, making real matches easy to distinguish from random background hits. BLAST uses a heuristic algorithm that seeks local as opposed to global alignments and is therefore able to detect relationships among sequences that share only isolated regions of similarity. (Altschul, et al. 1990).

BLAST Search Dialog Box

The BLAST search engine in Vector NTI initiates the search through a Vector NTI dialog box, launching the search through the NCBI (National Center for Biotechnology Information) server at the NCBI website (<http://www.ncbi.nlm.nih.gov/blast/blast.cgi>).

To open the BLAST Search window, select BLAST Search in the program group or folder where you installed Vector NTI. You will need to choose the server for the search in the small dialog box that opens (Fig. 32.1). (If you're not sure, select NCBI BLAST Server).

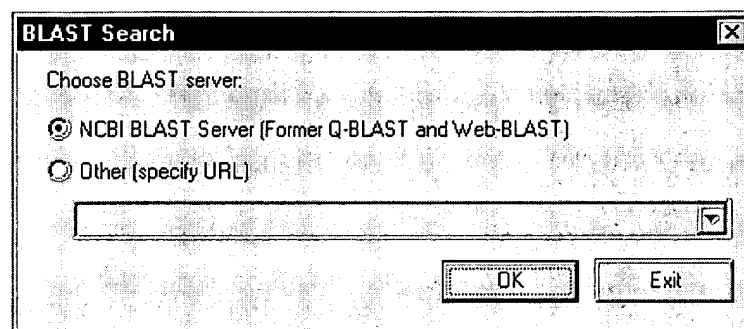


Fig. 32. 1 BLAST Search dialog box for server selection

Click **OK**, opening the following dialog box:

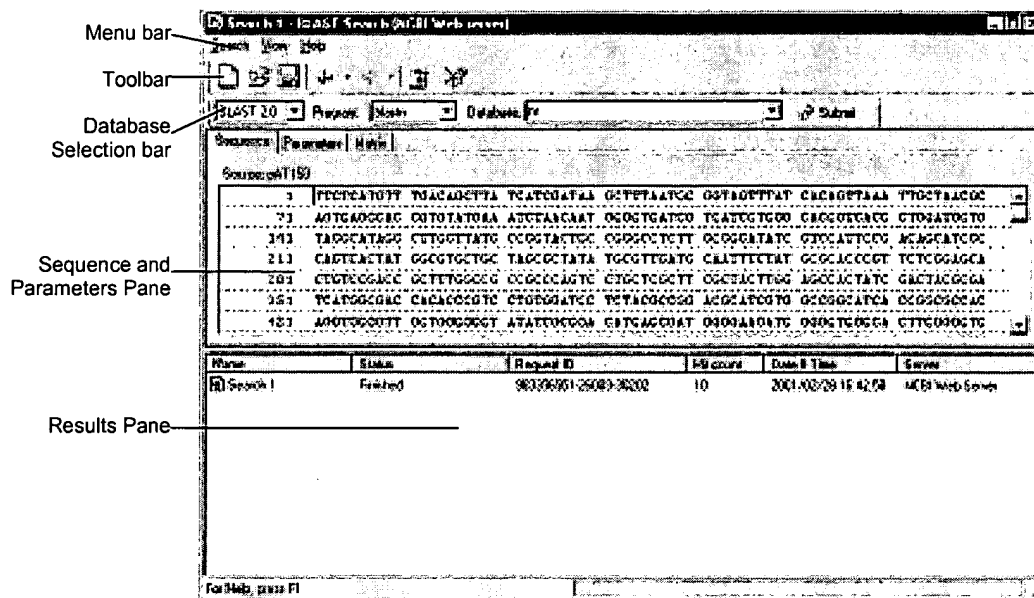


Fig. 32. 2 BLAST Search dialog box (Sequence tab)

The BLAST Search dialog box (Fig. 32.2) contains a small menu bar and toolbar with options relating to search, viewing and help functions. All of the toolbar buttons are described in Chapter 4, but they are displayed in this chapter as needed.

This dialog box is divided into three sections: the Database Selection bar, the Sequence and Parameters Pane and the Results Pane.

Database Selection Parameters

The default search engine in the Vector NTI Suite v.7.0 is BLAST 2.0. Because BLAST 2.0 searches are gapped by default, you must specify as explained below if you prefer an ungapped search.

Program - In the drop-down menu, specify the type of database search to be performed. The search options are summarized as follows:

- **blastn** - compares a nucleotide query sequence against a nucleotide sequence database
- **blastp** - compares an amino acid query sequence against a protein sequence database
- **blastx** - compares a nucleotide query sequence translated into all reading frames against a protein sequence database
- **tblastn** - compares a protein query sequence against a nucleotide sequence database dynamically translated in all six reading frames (both strands)

- **tblastx** - compares the six-frame translations of a nucleotide query sequence against the six-frame translations of a nucleotide sequence database. *This program cannot be used with the nr database.*

Database: In the drop-down menu, select the GenBank database type the query sequence is to be searched against. Many of the potential database type options are described below:

<i>Menu Identifier</i>	<i>Description</i>
nr	Peptide Sequence Database: All non-redundant GenBank sequences and CDS translations. Nucleotide Sequence Database: All GenBank+EMBL+PDB sequences (no EST, STS, GSS or phase 0, 1 or 2 HTGS sequences). No longer non-redundant.
EST (+ human, mouse, others)	Nucleotide Sequence Database: EST (Expressed Sequence Tags). Others includes all organisms except mouse and human
GSS	Nucleotide Sequence Database: Genome Survey Sequence, includes single-pass genomic data, exon-trapped sequences, and Alu PCR sequences.
HTGS	Nucleotide Sequence Database: Unfinished High Throughput Genomic Sequences.
Patents	Protein sequences from the Patent division of GenBank.
Yeast	Peptide Sequence Database: <i>Saccharomyces cerevisiae</i> protein sequences—genomic CDS translations. Nucleotide Sequence Database: <i>Saccharomyces cerevisiae</i> genomic nucleotide sequences.
Mito	Nucleotide Sequence Database: Mitochondrial DNA sequences.
vector	Nucleotide Sequence Database: Vector subbase of GenBank.
E. coli	Peptide Sequence Database: <i>Escherichia.coli</i> genomic CDS translations. Nucleotide Sequence Database: <i>Escherichia.coli</i> genomic nucleotide sequences.
month	Peptide Sequence Database: All new or revised GenBank CDS translation + PDB + SwissProt + PIR + PRF released in the last 30 days. Nucleotide Sequence Database: All new or revised GenBank+ EMBL+DDBJ+PDB sequences released in the last 30 days.

<i>Menu Identifier</i>	<i>Description</i>
Drosophila genome	Peptide Sequence Database: Drosophila genome proteins provided by Celera and Berkeley Drosophila Genome Project. Nucleotide Sequence Database: Drosophila genome provided by Celera and Berkeley Drosophila Genome Project.
PDB	Peptide and Nucleotide Sequence Database: Sequences derived from the 3-dimensional structure from Brookhaven Protein Data Bank.
kabat	Peptide and Nucleotide Sequence Databases: Kabat's database of sequences of immunological interest.
alu	Peptide Sequence Database: Translations of select Alu repeats from REPBASE. Nucleotide Sequence Database: Select Alu repeats from REPBASE.
dbest	Nucleotide Sequence Database: Database of GenBank+EMBL+DDBJ sequences from EST Divisions.
dbsts	Nucleotide Sequence Database: Database of GenBank+EMBL+DDBJ sequences from STS Divisions.
epd	Nucleotide Sequence Database: Eukaryotic promoter database.

Table 32. 1 GenBank database options

There are three tabs below the database selection section of the screen.

Sequence Tab

On this tab you can enter raw sequence (only) pasted from another application or select **Search > Open** and locate the file for a sequence you wish to submit. If the sequence is not in raw format, copy it to Notepad, which eliminates all characters except the text itself. Select and copy the sequence from the Notepad and paste it into the text box on the **Sequence** tab. If the BLAST Search module is launched from a Vector NTI Suite application, the active molecule sequence is pasted in the **Sequence** tab.

Parameters Tab

On the **Parameters** Tab (Fig. 32.3), you can define parameters for the BLAST search.

Note: The parameters you can set on this tab are almost identical to parameters for the Advanced BLAST searches at the NCBI website:

<http://www.ncbi.nlm.nih.gov/blast/blast.cgi>. For more information regarding these parameters than is detailed here, refer to the website.

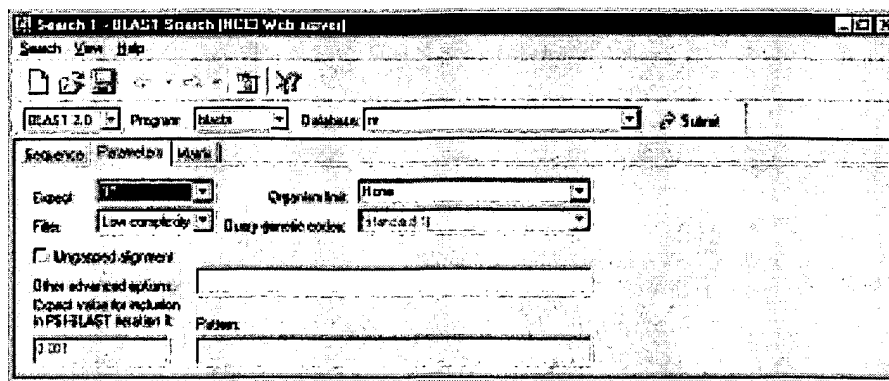


Fig. 32. 3 Parameters tab of the BLAST Search dialog box

Expect:

The statistical significance threshold for reporting matches against database sequences. The default value of 10 means that in a database of the current size, 10 matches would be expected merely by chance (stochastic model of Karlin and Altschul, 1990.) Hits showing a statistical significance greater than the Expect threshold are not reported. Increasing the E value above 10 produces a larger list with more low-scoring hits (chance matches). Lower expectation value thresholds are more stringent, leading to fewer chance matches being reported.

If your query peptide or nucleotide sequence is short, you might want to increase the Expect value. Because a short query is more likely to occur by chance in the database, even a perfect match can have low statistical significance and may not be reported. Increasing the E value lets you look farther down the hit list and see matches that would normally be discarded because of low statistical significance.

Filters:

- Low-complexity

This filter masks off segments of the query sequence that have low compositional complexity (as determined by the SEG program of Wootton & Federhen, Computational Chemistry, 1993). *Regions with low-complexity sequence can create problems in sequence similarity searching by producing artificial hits, sequences that are not truly related.* Such hits can produce high scores because of the presence of low-complexity regions.

- Human Repeats

This option masks Human repeats and is especially useful for human sequences that may contain these repeats. *This option is still experimental and may change in the near future.*

- Mask for Lookup

This option masks only for purposes of constructing the lookup table used by BLAST. The BLAST extensions are performed without masking. *This option is still experimental and may change in the near future.*

Ungapped Alignment:

Check this box if you prefer ungapped alignments. BLAST 2.0 allows gapped alignments, by default. Allowing gaps circumvents the problem of similar regions being broken into several segments. The scoring of gapped alignments tends to reflect biological relationships more closely.

Organism Limit:

This parameter lets you limit your database search to the most common organisms in GenBank, listed in this drop-down menu, or enter an organism name in the form “Genus species”, or leave the default “none”.

Query Genetic Codes:

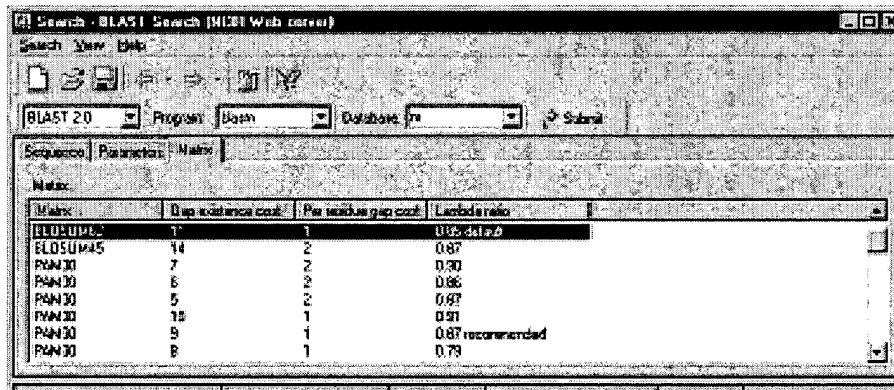
Set to a positive integer to select the genetic code that will be used by blastx and tblastx to translate the query sequence.

Expect value for inclusion in PSI-BLAST iteration 1:

This field is currently disabled. This option will be available in a future Vector NTI Suite release.

Matrix Tab

This tab permits you to specify the scoring matrix for blastp, blastx, tblastn and tblastx. The default matrix is BLOSUM 62. The valid alternative choices include PAM 30, 70, and BLOSUM 45 and 80. No alternate scoring matrices are available for blastn.



Matrix	Gap existence cost	Penalizes gap cost	Lambda ratio
BLOSUM62	11	1	0.12 default
BLOSUM45	14	2	0.87
PAM30	7	2	0.30
PAM30	6	2	0.66
PAM30	5	2	0.87
PAM30	10	1	0.91
PAM30	9	1	0.87 recommended
PAM30	8	1	0.79

Fig. 32. 4 Matrix tab of the BLAST Search dialog box, with gap penalties and lambda ratios displayed

Select among the substitution matrices listed, with their gap penalties and lambda ratios displayed (Fig. 32.4), which matrix to be applied to the BLAST search you are submitting.

BLAST Options

To specify technical settings for the BLAST Search, choose **View > Options**, opening the following dialog box (Fig. 32.5):

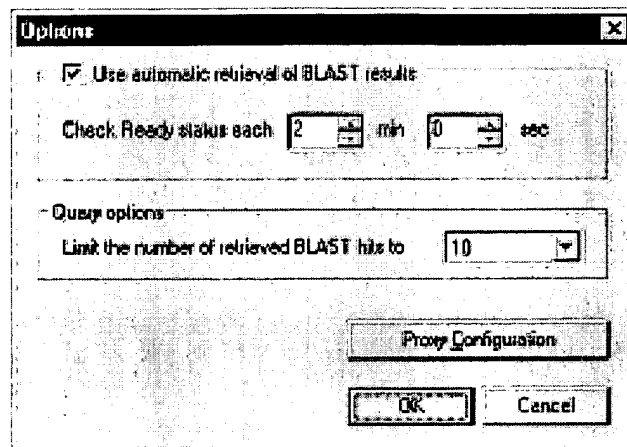


Fig. 32. 5 Blast Options dialog box

Complete your selections in the box. Press the **Proxy Configuration** button to set proxy server information (Fig. 32.6).

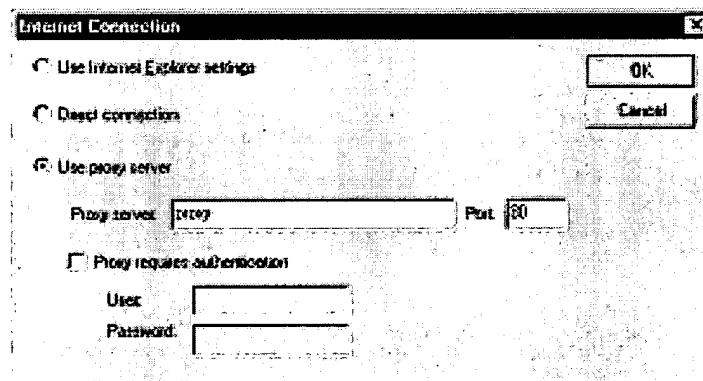


Fig. 32. 6 Internet Connection dialog box

Make sure that the proxy settings are filled in correctly; specifying whether or not your connection to the Internet should be performed via a proxy server. Enter the address and port number of that server. If your proxy requires a password, check the Proxy requires authentication box and enter the user name and password information.

Click **OK**, **OK**, returning you to the BLAST Search Viewer.

Press the **Submit** button () to launch the BLAST search.


BLAST Search Results

As soon as you have submitted your query, the search is numbered and listed in the Status Pane. This pane displays the following data about your search: data and time submitted, the status of the search (waiting, error, completed, etc. etc.), and the identification number assigned your query. Completed searches are listed also showing the number of identified hits in the response.

BLAST Viewer

Opening BLAST Viewer

The BLAST Viewer for reviewing BLAST search results can be opened by either of two methods:

1. Select the BLAST Viewer in the program group or folder where you installed Vector NTI. If you choose this method, it opens as an empty viewer. You can open previously saved BLAST results by clicking on the **Open** button () or by selecting **BLAST Results > Open**.
2. Double-click on a BLAST Search result entry in the BLAST Search dialog box. *See the beginning of this chapter.*

Elements of the BLAST Search Results Viewer

Blast Viewer displays the textual, sequence and graphical summary of the Blast search results (Fig. 32.7). It consists of a menu bar, and two toolbars and three panes: Text, Analysis and Alignment Panes.

All Vector NTI Suite toolbar buttons are summarized in Chapter 4 of this manual. The toolbar buttons are displayed as needed in this chapter, however. Many toolbar operations can also be launched from the menu bar or from a shortcut menu.

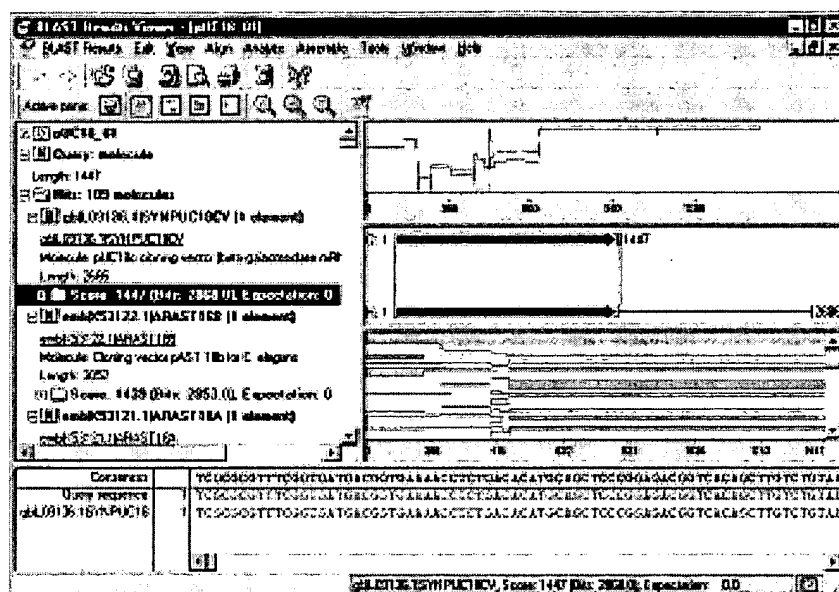









Fig. 32. 7 Blast Viewer

The BLAST Viewer is organized and managed like in the other display windows in Vector NTI Suite. Several BLAST Viewers can be opened in one workspace. You can navigate through the open display windows using the **Back** and **Forward** buttons (, ).

As in the other Vector NTI Suite applications, to apply a command in a given pane that pane must be active. The active pane can be toggled with the different panes buttons on the Pane Selection Toolbar (, , , , ) or by clicking anywhere in the pane you wish to activate. The keyboard strokes are F6 (cycling clockwise through the panes) and SHIFT + F6 (cycling counter clockwise). *Note that in this BLAST Search Viewer, each of the graphs in the Analysis Pane is assigned a separate Pane button.*

Two management features of the display window are designed to maximize viewing flexibility:

1. Select **View > Maximize Pane** to expand the size of an activated pane to full screen size. Select **View > Restore Layout** to return to the original pane layout.
2. Select **View > Edit Pane Layout** to open the Pane Manager for modifying the arrangement of the panes in the workspace. For more details, refer to Chapter 3.

The Status bar shows the Hit Element Score from the Text Pane folder for the selected hit element.

In addition, the following pane manipulations are outlined in Chapter 3:

- Re-sizing the Text Pane with the split bars or arrow keys
- Scrolling the Text Pane


BLAST Search Results

Text Pane

The Text Pane of BLAST Viewer contains a description of the query molecule, including common database information, a Feature map and a list of hit molecules. The Text Pane is linked to analyses graphs in the Analysis Pane and the Alignment Pane.

The following Text Pane manipulations are outlined in Chapter 3.

- Opening or closing folders
- Opening the folder shortcut menu

The first line of the Text Pane lists the name of the query molecule. Double-click on the line or click on the  at its left to open an Edit <molecule > dialog box. For details on editing the molecule see chapter 20.

The second entry in the Text Pane contains information about the query sequence such as the molecule length. This is followed by a folder of the Hit molecules, also displaying the number of hits. Click on the + at the left of the folder to open all of the subfolders.

The hit molecules themselves are links to the NCBI website. If you click on the link, that prompts the downloading of the molecule, thus opening it in a Molecule Display window.

For each hit molecule, the following information is provided:

- Its ID code and the number of hit elements (regions of local similarity) in the molecule.
- A brief summary relating to the matching region of the hit molecule to the query sequence and the length of the matching region
- Score Folder(s) corresponding to the number of hit elements

Hit Element Description

Double-Click on a Score Folder for a particular hit element to open it. The folder displays the statistical results for the hit element such as its expectation value, identities, etc.

Selecting a Score Folder for a hit element locates *all* of the hit elements for that hit molecule in the Analysis Pane. However, just the hit element corresponding to the selected folder is displayed in a colored mode. As you click on the other Score Folders in the Text Pane, they are each highlighted in the Analysis Pane, in turn.

The statistical results in the Score Folder are described as follows:

- **Score + (Bit Score):** These indicate how close the identity of the match is to the query sequence.
- **Expectation:** This value reflects the likelihood that the similarity between the sequences would occur by chance when searching a database of a particular size. A zero or extremely low number suggests that the match is so perfect that it is extremely unlikely that the similarity would occur randomly. The E value describes random background noise exists for matches between sequences.
- In a BLAST search, an E value of 1 assigned to a hit can be interpreted as meaning that in a database of the current size one might expect to see 1 match with a similar score simply by chance. *For more detail about this score, see the BLAST parameters section at the beginning of this chapter.*
- **Identities:** the ratio (and percentage) of matching residues in the hit elements. The numbers n/n refer to the number of identical residues out of the number of matches in the hit element. This is important to consider when determining the significance of this statistic. A high identity percentage may mean nothing if a low number of nucleotides is being compared.
- **Positives:** the ratio (and percentage) of similar residues in the hit elements
- **Query:** The position numbers in the query sequence matching that of the hit element are indicated by the start and end positions of the figures, such as 57[bp] – 93[bp]. The strand that corresponds to the hit element is also shown.
- **Hit:** The position numbers in the hit sequence matching the query sequence are indicated by the start and end positions of the figures, such as 42[bp] – 88[bp]. The strand that corresponds to the hit element is also shown.

Analysis Pane

The Analysis Pane, displaying the graphical summary of the BLAST Search results, consists of three panels: 1) the upper graph displays the Sequence Profile and the Hit Distribution, 2) the center graph displays the Query-Hit Alignment, and 3) the lower graph displays the Hit Map graph.

Sequence Profile Graph (upper pane)

The Sequence Profile Graph depicts how well the particular regions of a query molecule are represented in a database it was searched against, allowing “unique” and “shared” regions of a query molecule to be easily identified. Each residue of the query sequence is assigned a value indicating the position-specific representation of this residue in a database.

The profile is calculated as follows: each residue is traced into all hit elements in which it is included. The corresponding residue substitution score from a hit element is multiplied by a logarithm of an expectation value of a particular hit element and the resulting value is summarized over all hit elements. Each step in the resulting graph represents the profile of each sequence residue.

If the database search utilized a translation of the original molecule as an intermediate step (tblastx and blastx), the sequence profile graph is generated for all possible translation frames.

Hit Distribution Graph (upper pane)

Each residue in the query sequence is traced to all hit elements in which it is included, and 100 percent identical. The number of hit elements per residue are tallied (summed up) and plotted across the molecule.

Query-Hit Alignment Graph (center pane)

Each element on the Hit Distribution Graph positions the local similarity regions on the query and hit molecules. The upper scale represents the query sequence while the lower scale represents the currently selected molecule in the hits list. The hit element corresponding to the currently selected Score Folder (Text Pane) is shown in color on the map, while the other hit elements for the molecule are grayed out. To select another element in the graph, you must return to the Text Pane and select a new Score Folder.

Hit Map Graph (lower pane)


The Hit Map graph provides a graphical overview of the hit sequences aligned to the query sequence. The X-axis on this map represents residues along the query molecule. Each bar drawn above the X-axis represents a molecule (or molecule fragment) that matches the query sequence. The position of each bar relative to the linear axis of the query allows users to see instantly the extent of the hits that align with single or multiple regions of the query.

Hit molecules are represented in decreasing order of similarity with the most similar hits shown at the top and the least similar shown at the bottom. If one hit molecule contains multiple hit elements, they are represented along the same Y-axis position, irrespective of similarity scores.

To view the Name, score, bits and E-value of a hit, pause the cursor over the hit element bar on the graph. Clicking on a hit element bar selects the hit element in the Text Pane. This also changes the bar's color to indicate the selected hit and displays the alignment between the query and the hit sequences in the Query-Hit Alignment pane.

Zooming In and Out

Enlarging or reducing regions of the Analysis Pane is possible using the Zoom buttons. Click in the pane for the graph to be resized. If you Zoom In sufficiently, you can see the individual residues on the X axis. For more detailed about using the Zoom buttons, refer to Chapter 3.

To view a specific selected region in one of the graphs, select Zoom to Selection from the shortcut menu. To return to the normal mode, click on the **Zoom to Fit** button ()

Note: In the Analysis Pane, only the upper and lower graphs can be resized.

Selecting Regions on the Analysis Pane

Regions of the graphs can be selected in the Analysis Pane by holding down the left mouse button then dragging through the sequence. Selected regions are displayed concurrently on other graphs and the Alignment Pane, (only if the Score Folder for the hit element corresponding to the selected region is selected in the Text Pane.)

Hits listed in the Text Pane that correspond with the selection in the Analysis graphs can be highlighted using the **Select Hits** function. To use the Select Hits function, highlight the area of interest on the Analysis Pane graphs and choose **Edit > Select Hits** from the menu. The Select Hits dialog box appears (Fig. 32.8):

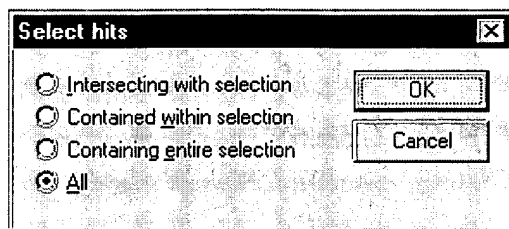


Fig. 32. 8 Select Hits dialog box

There are four choices for mapping the selection to the hits in the Text Pane:

- **Intersecting with selection** – hits that contain any part of the selected area.
- **Contained within selection** – the entire length of the hit matches within the selection.
- **Containing entire selection** – hits contain the entire selection and, possibly, more.
- **All** – hits with any of the above matching are found.

Select the radio button for the intersection option of your choice and press the **OK** button. Hits corresponding in the Text Pane corresponding to the selection in the Analysis Pane and the selected intersection option are highlighted.

Viewing/Changing Parameters of the Analysis Graphs

The graphs in the Analysis Pane display the values averaged in a window of a specific length sliding along the x-axis of the graph by a specific step.

To view and modify the parameters of the Sequence Profile or Hit Distribution plots, activate the upper graph pane by clicking on its Pane button, or click in the upper pane. Select **Plot Setup** from its associated shortcut menu, opening the Plot Setup dialog box:

Analysis tab: Count Window Size is the length of the sliding window; the larger the Count Window Size, the smoother the curve. Step Size is the width of each step on the plot.

Sequence Profile/Hit Distribution tabs: Modify the plot color and select the graph display mode from Bar, Boolean, or Linear.

To view and modify the Hit Map graph properties, activate the Hit Map pane by clicking on it or by pressing its activation button on the toolbar. Right-click on the Hit Map graph pane and choose **Properties** from the shortcut menu or choose **Edit > Properties** from the menu to launch the Properties dialog box. In the properties dialog box, you can change the display color for hits and the selected hit and adjust the size of the graphic hits display.

Alignment Pane

The Alignment Pane displays the alignment of the hit element selected in the Text Pane and the corresponding region on the query sequence. The query molecule, the currently selected hit element and the resulting consensus sequences are shown.

Moving Around the Alignment

The Alignment Pane has its own horizontal scrollbar, if the sequences are sufficiently long to warrant them. The sequence names remain stationary at the left in the Alignment Pane no matter how the sequences are repositioned horizontally. The position number of the left-most residue visible in each sequence is displayed immediately following each sequence name.


Selection Techniques

Select molecules in the Alignment Pane by clicking on their names.

To select regions of the nucleotide or amino acid sequence alignments, use the click and drag technique with the mouse. *Selection applies only on the sequence of the currently selected molecule.* The selection is also shown on the graphs in the Analysis Pane.

Alignment Properties

To change a default set of alignment display parameters, From the Alignment Pane, select

Properties from the shortcut menu or click on the **Properties** button (). The Alignment Display Setup dialog box opens with three property tabs: Consensus Calculation, Similarity Tables, and Color Setup.

Consensus Calculation

A consensus sequence is a theoretical representative nucleotide sequence in which each nucleotide represents either the residue seen most frequently at that same site in aligned sequences, or is selected by other criteria. The Consensus Calculation tab specifies how the consensus sequence, displayed as the bottom sequence in the Alignment Pane, is calculated in BLAST Viewer.

On the Consensus Calculation Tab, you can select among the following parameters:

- Consider only identical residues
- Ignore gaps in consensus calculation
- Specify a sequence to use as the consensus sequence. In this case, select one of the sequences listed (those used in the alignment).
- Residue fraction

If the **Use sequence as consensus** checkbox is checked, the Consensus will not be calculated but the sequence selected in the listbox below the checkbox will be used as the consensus.

Similarity Table

The Similarity Table allows you to review or to define residue similarity information and values necessary for calculation of the alignment quality profile. Each residue pair can be defined as being strongly or weakly similar to each other. The table is editable only for amino acid residues.

Color Setup


The Color Setup tab allows you to review or modify the current color setup scheme for the Alignment Pane:

Color Scheme	Interpretation
black on window default color	non-homologous residues
blue on cyan	consensus residue derived from a block of similar residues at a given position
black on green	consensus residue derived from a single conservative residue at a given position
red on yellow	consensus residue derived from completely conservative residues at a given position
green on window default color	residue weakly homologous to consensus residue at given position

Table 32. 2 Color Setup tab interpretations of color scheme

Saving BLAST Search Results

To save BLAST search results into the database for future retrieval, select **Blast Results >**

Save As or press the **Save As** button (). Select either of the two tabs in the Save As dialog box (Fig. 32.9), depending on your preferred destination. The results can be saved in the Vector NTI database or as a BLAST result file.

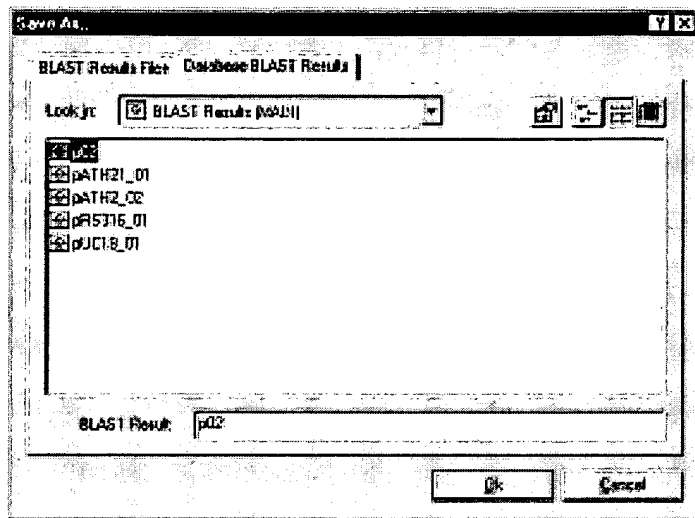


Fig. 32. 9 Save As dialog box, where results can be saved in the Vector NTI database or as a BLAST result file

Select the subbase or folder (and file format for Results File) and enter a name for the results (Fig. 32.10).

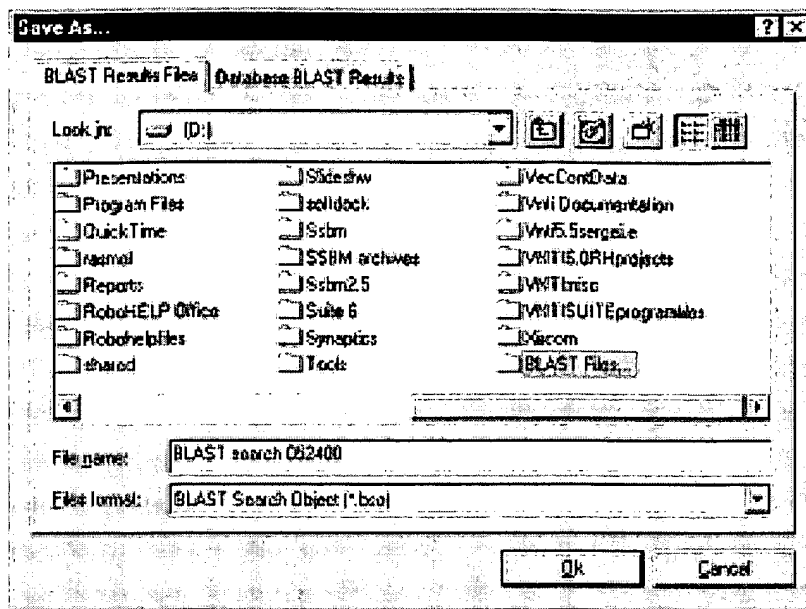


Fig. 32. 10 Selecting the subbase or folder, file format, and name for Results File

Click **OK** to save the BLAST results as a new database object, associated with the query molecule. You can open search results for the query molecule from the Molecule Viewer window.

To save individual BLAST hits into the VECTOR NTI database, select the desired search hits and choose **Save Selected Hits** from the shortcut menu, opened by a right click on the molecule name. Hits will be saved to corresponding molecules and can be viewed later in the Molecule Viewer.

To open individual BLAST hits into VECTOR NTI, select the desired search hits and choose **Open Selected Hits** from the shortcut menu, opened by a right click on the molecule name. The molecule(s) can then be saved to the VNTI Database or to files on the hard drive.

Opening Query Molecule with Hits Displayed as Features

You can open the query molecule that was used for the BLAST search in Vector NTI with the BLAST hits displayed on the molecule as features. If desired, you can then save the annotated query molecule to the VNTI Database.

To open the query molecule in VNTI with the hit sequences displayed as features, choose **Edit > Open Query Molecule with Hits as Features** command from the menu. A dialog box appears allowing you to choose the feature type you want to use. Choose the desired feature type, check the box to save existing query molecule features, if desired, and press

the **OK** button. The query molecule opens with the BLAST hits displayed as the chosen feature type.


In the Text Pane of the new molecule, query hits are listed in the chosen feature type folder in order of their occurrence on the query molecule. Hit feature names follow the convention "Feature Type_#1 / #2" where

- Feature Type is the type of feature that was chosen when the Open Query Molecule with Hits as Features command was executed
- #1 is the hit molecule number according to its listing in the BLAST Viewer and
- #2 is the hit element number of the hit molecule.

The new molecule can then be saved to the VNTI Database or to a file on the hard drive.

Creating a New Database Subbase for BLAST Search Results

To create a new database subbase for storing BLAST search results, in the BLAST Viewer,

press the **Database** button () , opening Vector NTI Suite's Database Explorer. The Explorer opens to the BLAST Results table. To create a new subbase for storing BLAST Search results, follow the instructions given in Chapter 18.

Other BLAST Viewer Features

The following features can be performed in BLAST Viewer. Details are given in Chapter 3.

- Copying selected molecule IDs to the clipboard
- Exporting data to external programs
- Printing from the BLAST Viewer

Chapter 33 AlignX Blocks

Introduction

AlignX Blocks is a program for locating, analyzing, and editing blocks of localized sequence similarity among multiple *protein* sequences and linking them into a composite multiple alignment.

The following definitions are important in the AlignX Blocks application:

- A *Block* is a set of sequence segments of the same length taken from several sequences and aligned without gaps.
- A *Local Alignment* is an alignment that includes only the most similar regions (even fairly small) of the sequences under consideration. Such regions may span several sequences.

A Blocks project, including molecules, settings and blocks generated in AlignX Blocks can be saved as a file. This enables you to restore the project later to continue working with it.


Opening AlignX Blocks:

To open AlignX Blocks, select it from the program group or folder where you installed the Vector NTI Suite.

Features of the AlignX Blocks Workspace

When AlignX Blocks is first opened, the display window is empty. As molecules are added to an AlignX Blocks project, they are listed in the Text Pane. After performing a Block Search on a group of molecules selected in the Text Pane, blocks results appear in the other panes.

You may want to open a demo project into the workspace before you review its features.

Select **Project > Open** or press the **Open** button (). Find the Vector NTI Suite folder, probably located in Program Files\Vector NTI\Demo Project, and open the Protein.bpr file.

The AlignX Blocks interface (Fig. 33.1) consists of a menu bar, three toolbars, an AlignX Blocks display window, with shortcut menus specific for each pane. The AlignX Display window includes four panes.

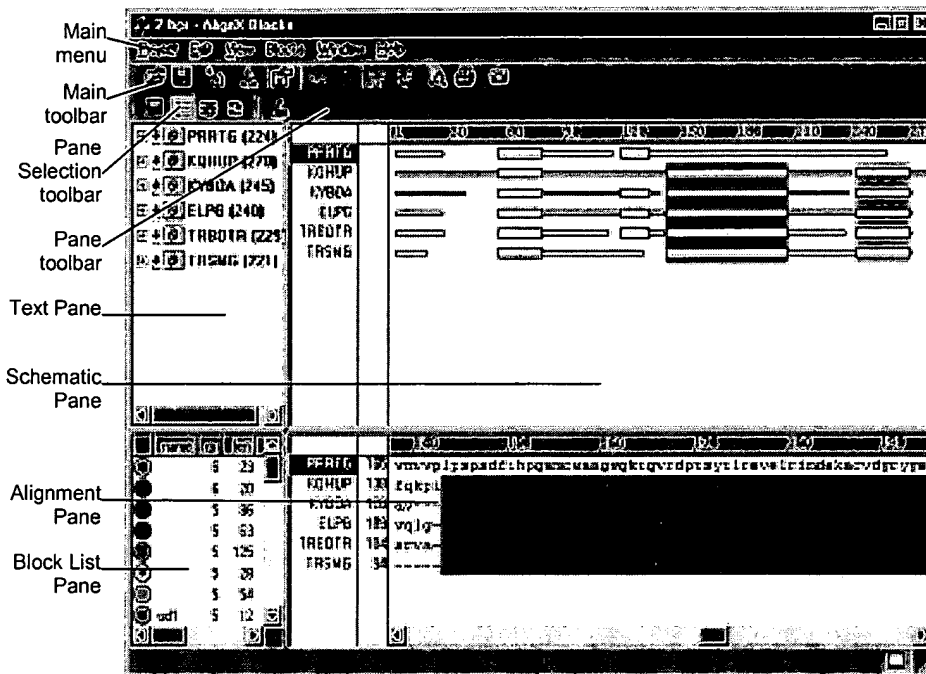






Fig. 33. 1 AlignX Blocks interface

AlignX Blocks has three toolbars: a Main Toolbar, the Pane Selection Toolbar and a Pane Toolbar. All Vector NTI Suite toolbar buttons are summarized in Chapter 4 of this manual. As you are introduced to various operations in this chapter, however, the toolbar buttons you will use are displayed appropriately. Many toolbar commands can also be launched from the menu bar or from a shortcut menu.

The AlignX Display window is divided into four panes: a Text Pane, the Block List Pane, an Alignment Pane and a Schematic Pane. All panes are separated by split bars that allow re-sizing of the panes. Scroll bars appear when the contents of a pane exceed the viewing area, facilitating easier viewing of pane contents.


As in the other Vector NTI Suite applications, to apply a command from the main menu in a given pane (Print, Print Preview, Camera), that pane must be active. The active pane can be

toggled with the **Switch Panes** ( ,  ,  , ) buttons in the Pane Selection Toolbar or by clicking anywhere in the pane you want to activate.

You can maximize any active pane to fill the entire display space by selecting **View > Maximize Pane**. Select **View > Restore Layout** to restore the original pane size.

You can modify the pane arrangement in Pane Manager. Select **View > Edit View Layout** to open the Pane Manager. For more details, refer to Chapter 3.

Text Pane

To activate the Text Pane, click on the **Text Pane** button () on the Pane Selection Toolbar. The Text Pane contains a thorough description of the molecules included in the AlignX Blocks project. The information is organized in folders, named by molecule. Each top level (molecule) folder contains the following subfolders:

<i>Folder</i>	<i>Contents</i>
General description	Molecule type, length, etc.
Comment	Arbitrary text of any length associated with the molecule
Standard fields	GenBank/SWISS-PROT-like fields: keywords, division, original accession numbers, etc
References	Bibliographic references (in GenBank/SWISS-PROT format)
Proprietary fields	User defined fields (for molecules which came from Vector NTI)
Analysis	Results of common physiochemical analyses
Feature table	List of molecule features

Table 33. 1 AlignX Display window Text Pane folders



Folders and sub-folders can be opened with a double-click or by clicking on the + to the left of the folder name.

For more information regarding the following Text Pane manipulations, refer to Chapter 3.


- Using the scrolling bars
- Expanding and collapsing folders
- Copying pane contents to the clipboard

Finding Signals on Schematic and Alignment Panes

Signals or features listed in the Feature map subfolder in the Text Pane can be found in Schematic and Alignment Panes. This feature is only enabled if the molecules are involved in the blocks located in the Blocks Search, indicated by a blue arrow. To find the positions

of the signal in these panes, select the feature line, indicated by the tag icon () and press the **Find** button () from the Text Pane toolbar or select **Edit > Find**. The *Find* command is disabled if the selected line is not a signal with non-zero boundaries.

Block List Pane

All of the Blocks, found in a Block Search or manually defined, are shown in the Block List Pane (Fig. 33.2). To activate this pane, click within the pane or click on its button .








	name	m	len	MP-score	SP-score	Signif.	Offset
		5	210	970.7	9707	✓	1
		4	215	954.0	5724	✓	151
		4	122	376.3	2258	✓	21
		3	44	119.0	357	✓	125
		3	52	39.7	119	✓	117
		3	52	39.7	119	✓	117
		3	13	32.0	96	-	22

Fig. 33. 2 AlignX Display window Block List Pane

Each line in the pane represents one block. The following columns (fields) are defined for the block:

- **Color representation:** A color is assigned to each block automatically from the list of available colors (defined in Project properties). The first block in the list gets the first color, second block – the second color, etc. If there are more blocks than colors the same colors are used again.
- **Name:** Initially, blocks found in a search have no name. Manually defined blocks are automatically named “ud1”, “ud2”, etc. You can name or rename a block in the Block Edit dialog.
- **m:** Number of sequences in a block
- **Length:** Length of the block, that is, the length of the sequence segments in the block. Like segments for all the sequences in a block have the same length.
- **MP-score:** Mean Pairwise Score, calculated by dividing the SP-score of a block by the number of possible pairwise combinations of component sequence segments.
- **SP-score:** Sum of the Pairs Score, composed of several sequence segments. Scores are calculated for all possible segment pairs. The sum of all of the scores is the SP-score.
- **Significance:** Biological significance (see “Methods for Assessing the Statistical Significance of Molecular Sequence Features by Using General Scoring Schemes” by Samuel Karlin and Stephen F. Altschul. 1990).
- **Offset:** Minimal offset of the segment in the block.

Block Selection and Highlighting

There are two methods of selection in the *Block List Pane*, each offering different functionality. Both types of selection work independently and do not affect each other.

1. **Single selection:** Because this selection enables actions within a block, only one block at a time can be selected with this method. Click on the block line outside of the colored circle area. The highlighted selected block is shown concurrently in the Schematic and Alignment Panes. To deselect the block, click on the block in the Schematic or Alignment Panes
2. **Multiple selection:** This selection method allows no actions within blocks, but is only for viewing them. Select one block by clicking within the colored circle area. Press SHIFT + CLICK within circles to select a range of several blocks. Press CTRL + CLICK within circles to select more than one non-contiguous block. To deselect the blocks, repeat the clicks on the selected circles.

Blocks selected in this way are indicated by the black dot in the center of the colored circle in the Block List Pane. The colored blocks on the Schematic and Alignment Panes correspond to the selected circles, distinguishing one from the other. Mouse clicking within Schematic and Alignment Panes does NOT remove this kind of selection.


Sorting Blocks in the Block List Pane

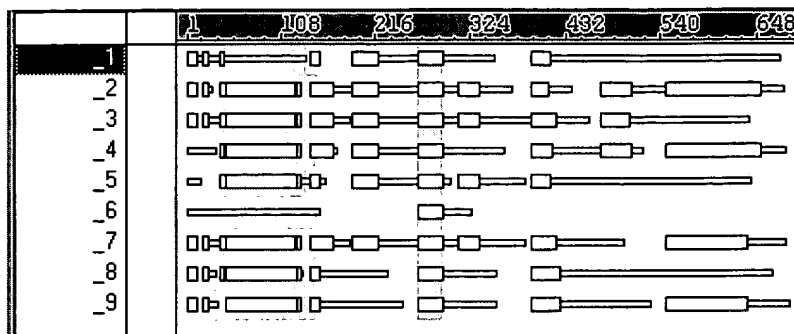
Initially blocks are sorted by the number of sequences in the block (m-column). The blocks order in the Block List Pane can be changed, however, by sorting. Click on a column header to sort; repeat the click to reverse the sorting order. Sorting is unaffected by selection of one or more blocks.

Removing Blocks

To delete a selected block, choose **View > Delete Block** or the corresponding command from the Shortcut menu. You can confirm or cancel the deletion.

Schematic Pane

This pane is designed for a schematic representation of blocks to facilitate analyses of their position and relationship. Click in the pane or on the **Schematic Pane** button () to activate this pane.



Molecules included in the Block Search operation as well as manually added from the Text Pane are displayed schematically as stretched rectangles (Fig. 33.1). A schematic representation of one sequence occupies each row, with the name of its sequence displayed to the left. The upper row of the pane is a ruler. Both molecule name and its sequence can be scrolled in the vertical direction. There is no horizontal scrolling bar for this pane because the display is calculated such that the longest rectangle fits the pane width.

You can change the order of the molecules in the pane by dragging a molecule with the DRAG + DROP technique. The order of the molecules in the Alignment Pane reflects the changes.

To select molecule(s) in the Schematic Pane, click on the name(s). Selections are highlighted concurrently in the Alignment Pane. To select a region in the Schematic Pane, click within the pane and drag a rectangle.

Note: In this pane, you can only select regions; you cannot select blocks.

Alignment Pane

The Alignment Pane (Fig. 33.4) is designed for viewing and analyzing sequential

information. To activate it, click within pane or click on the **Alignment Pane** button () of the Pane selection toolbar.

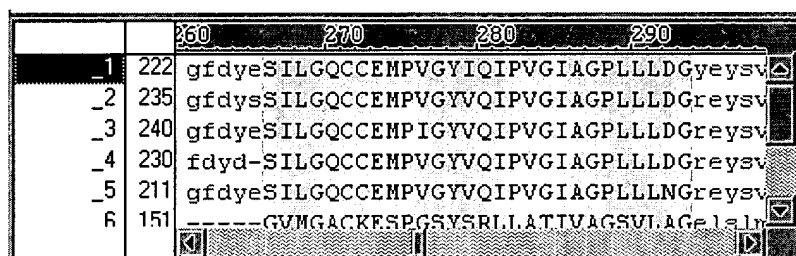


Fig. 33. 4 AlignX Display window Alignment Pane

Molecules included in the Block Search operation as well as those manually added from the Text Pane are displayed as sequences in the Alignment Pane. The name of the molecule and current position within the sequence appears to the left of its sequence. The upper row of the Alignment Pane contains a ruler with a scale of residues.

The Alignment Pane has its own vertical and horizontal scrollbars. Both a molecule name and its sequence can be scrolled in the vertical direction. When scrolled horizontally, the sequence names remain stationary at the left of the Alignment Pane. The number to the left of the sequence shows its current position.

Dragging the molecule (up or down) by the name and dropping it on the desired place can change the order of the molecules in the pane. The order of the molecules in the Schematic Pane is changed to reflect the order in the Alignment Pane.

Selection in the Alignment Pane

To select a molecule(s) in the Alignment Pane, click on its name. This selection is selected concurrently in the Schematic Pane as well.


To select a region in the Alignment Pane, click and drag within the pane. The region inside the tracking dotted rectangle is selected except for the “gapped” (or empty) areas. To combine a new selection with a previous selection, press the CTRL button while starting the new selection. To modify a selection, drag the edge of the selection to the right or left. Selections are displayed concurrently in the Alignment and Schematic Panes.

AlignX Blocks Projects

A set of molecules, options and blocks opened and being worked on is called a AlignX Blocks project. AlignX Blocks accepts following file formats: GenPept, SWISS-PROT, FASTA and Vector NTI archives.

Opening an Existing Project


When AlignX Blocks is launched, a new, empty project is created. To open an existing

project, choose **Project > Open** or click on the **Open Project** button () on the Main toolbar. Select the desired project name in the File Open dialog box. *AlignX Blocks projects have a “*.bpr” file extension by default.* Select the desired file and click **Open**. If the file is a valid AlignX Blocks project, information about molecules, properties and blocks (if any) will appear in appropriate panes. An alternative way to open an existing project is to drag and drop the project file on the AlignX Blocks workspace.

Creating a New Project

To create a new, empty project, select **Project > New**. If appropriate will be prompted to save any changes in the currently opened project first. If you select **Cancel**, work may continue on the opened project. If you select **Yes** to save changes or **No** to disregard changes, the current project is closed and the workspace emptied.


Adding Molecules to the Project

To add molecules to a current project, press the **Add Files** button () or select **Project > Add Files**. *Only protein molecules in acceptable format can be added to the project.* Molecule(s) added to the project are listed in the Text Pane.

You can also add molecules to the project using the drag and drop technique if they are of an appropriate file format and molecule type. If the program fails to define a file's format, it will be suggested the file be imported as a raw sequence. Imported sequences appear in the Text Pane with the names: NONAME, NONAME#2, NONAME#3, etc.

Block Search

To search for blocks, select two or more molecules in the Text Pane and click **Search for**


Blocks button () or choose **Blocks > Search for Blocks**. The Block Search progress dialog box opens, letting you monitor the search progress. Press the **Cancel** button to interrupt the current search. Blocks located in the search (if any) are shown in the Block List Pane, sequences of the selected molecules are displayed in the Alignment Pane and schematic representations of the sequences in the Schematic Pane.

Adding Sequences to Block Sequence Space.

The term Block Sequence Space (BSS) designates all the sequences that are displayed in the Schematic and Alignment Panes. Usually sequences are added to BSS as a result of a Block Search. Selected sequences appear in both Schematic and Alignment Panes regardless of whether or not either any block includes this particular sequence. To add a sequence currently not present in a BSS, select **Add Sequence** from the shortcut menu opened from the Text Pane. *If a sequence already is included in a BSS, this menu item is disabled.* A sequence added using the **Add Sequence** command is also marked with blue arrow in the Text Pane.


Defining a Block


In AlignX Blocks, you can search for blocks automatically or define blocks manually. To define a block, select a region within the Alignment or Schematic Pane and click the **Define**

Block button () , select **Blocks > Define Block** or the corresponding command from the Shortcut menu. All the segments of the newly defined block will have an offset number defined by the selection, and the length equal to the minimal segment length. All previous selections are overwritten and the new block is selected in all three panes. Manually defined blocks are named automatically as "ud1", "ud2", etc, but they can be renamed later.

Linking and Unlinking



Linking means aligning the currently selected sequence segments and creating "links" between the residues appearing in each effected column. When residues are linked, they remain in the same column despite any shifts that occur elsewhere in the alignment. Linked residues are shown in upper case in the Alignment Pane and as wider rectangles in the Schematic Pane.

To link, first select sequence segments within the Alignment or Schematic Panes, or select a block in the Block List pane. Press the **Link** button () or select **Blocks > Link** or the corresponding option on the shortcut menu.

To unlink selected sequence segment(s), press **Unlink** button () or select **Blocks > Unlink** or the corresponding command on the shortcut menu. Unlinking selected segment(s) can cause segments in other sequences to also become unlinked.


To unlink all segments of all sequences, select **Blocks > Unlink All**.

When you perform Link functions, the previous state is saved in the history list.

The **Undo Link** button () and **Redo Link** button () buttons on the Main toolbar allow navigation through the history list. Information about links is saved in saved in an AlignX Blocks project and applied to Alignment Pane next time you open the project.

Color Setup

Colors for displaying linked and unlinked areas in the Alignment Pane can be changed in the Link Color dialog box (Fig. 33.5). To modify the Link Color, click on the **Link Color**

button () or select **View > Color Setup** or the corresponding command from the shortcut menu, opening the Link Color dialog box.

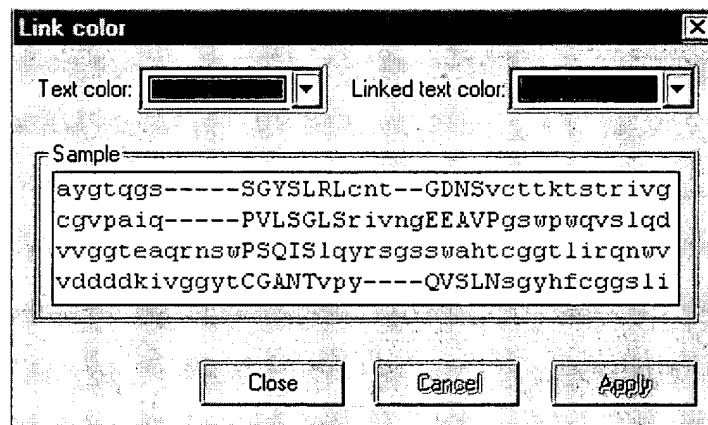



Fig. 33. 5 Link Color dialog box

This dialog box lets you select the background sequence color (Text color) and Linked text color. Select the appropriate color(s) and click **Apply** to preview the selections. Click **Close** to apply the changes and close the menu.

For more information regarding linking and unlinking, see the previous section.

Block Editing

To edit a block, press the **Edit Alignment** button () or choose **Blocks > Edit Block** or the corresponding command from the shortcut menu, launched by right clicking on the desired block or double click on the desired block. This opens the Edit Block dialog box (Fig. 33.6).

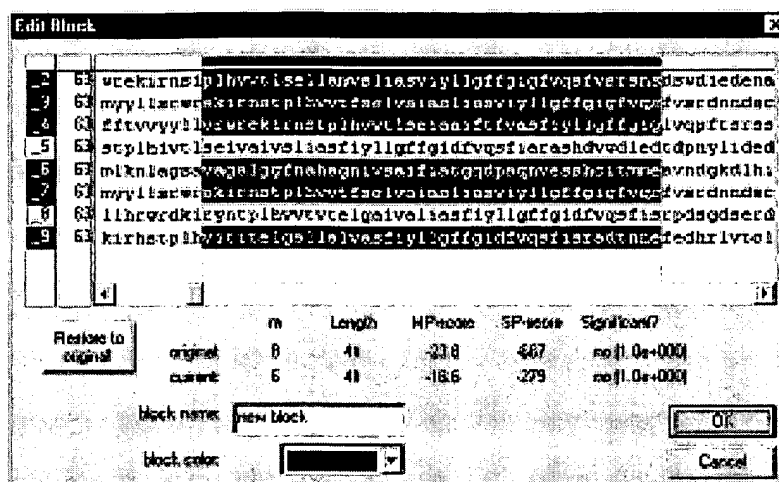



Fig. 33. 6 Edit Block dialog box

In the Block Editor:

1. Remove sequences from a block by clicking on their number (at the far left).
2. Narrow or widen a block by dragging the line separating it from the rest of the sequence. All changes in block statistics are immediately displayed in the current line (in the section below the Alignment Pane).
3. Name or rename a block in the block name text box.
4. Change a block's color in the block color dropdown menu.
5. Press the **Restore to original** button to undo all changes.
6. After changes are saved, the Block List, the Schematic and Alignment Panes are updated.
7. The block statistics in Block Editor are described as follows:
 - **m**: number of sequences in a block
 - **Length**: Length of the block, that is, the length of the sequence segments in the block. Like segments for all the sequences in a block have the same length.


- **MP-score:** Mean Pairwise Score. The MP-score is calculated by dividing the SP-score of a block by the number of possible pairwise combinations of component sequence segments.
- **SP-score:** Sum of the Pairs Score, composed of several sequence segments. Scores are calculated for all possible segment pairs. The sum of all of the scores is the SP-score.
- **Significant?:** Significance of a block. Standard P-value (Karlin, Altschul, 1990)

Deleting a Molecule

To delete a selected molecule from the project, press the **Delete from Project** button () or choose the Delete From Project from the shortcut menu.

If a selected molecule participates in one or more blocks, you will be asked to verify the deletion. *All blocks it participates in will be dismissed.* If you confirm the deletion, the specified molecule and all related blocks are removed from all panes.

AlignX Blocks Parameters

To review or modify AlignX parameters setup, click on the **AlignX Blocks Setup** button () or select **Blocks > AlignX Blocks Setup**. This opens the AlignX Blocks Parameters dialog box (Fig. 33.7).

On the three tabs, review or modify the following parameters:

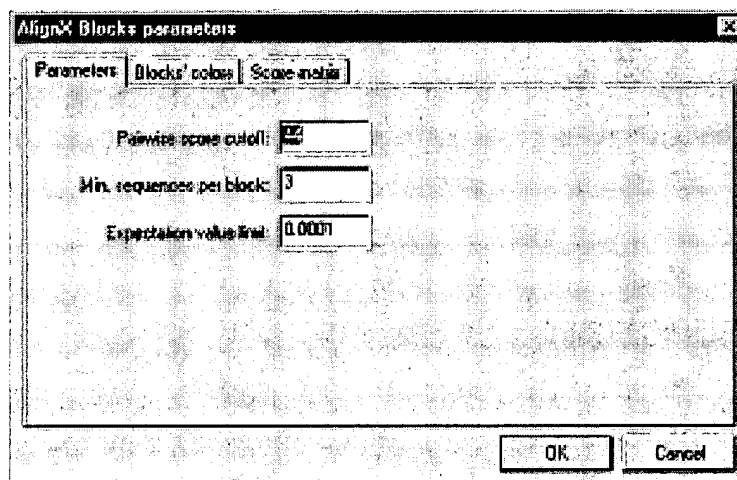


Fig. 33. 7 AlignX Blocks Parameters dialog box (Parameters tab)

Parameters Tab:

- **Pairwise score cutoff:** the minimum segment pair score needed to mark a diagonal
- **Min. sequences per block:** the minimum length of sequence that block must span in order to be reported
- **Expectation value limit:** the maximum threshold value required to make block significant

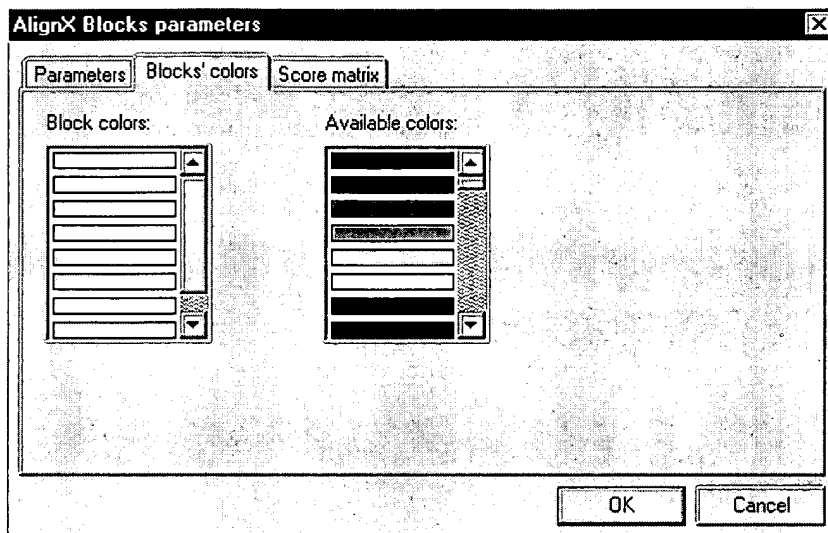


Fig. 33. 8 Blocks' Colors tab on the AlignX Blocks Parameters dialog box

Blocks' Color Tab:

To change Block color(s), select the appropriate color from the palette then click **OK**.

There are two color listboxes on this tab: **Block colors** selected for this project and **Available Colors (Fig. 33.8)**. Double click on any color from the Available Colors list to add it to the Blocks Colors list. To remove a color from the current block double click on the color in the Block colors list.

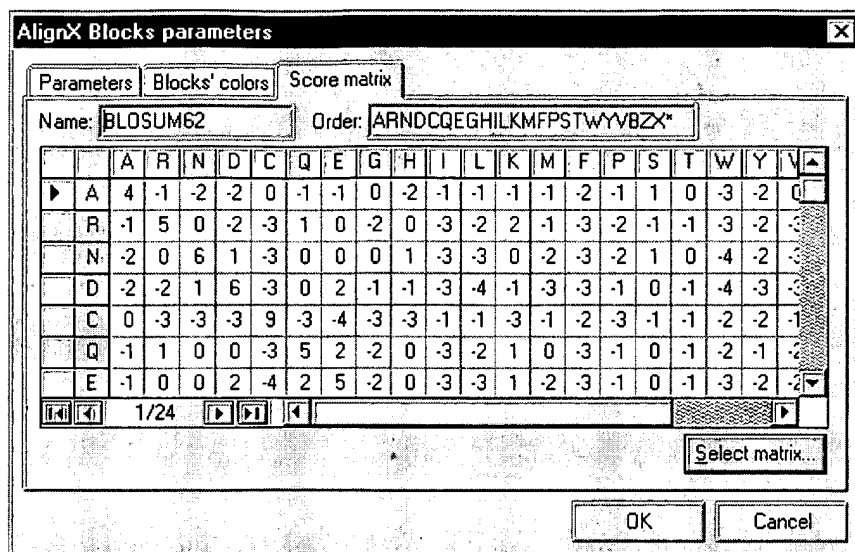


Fig. 33. 9 Score Matrix tab on the AlignX Blocks Parameters dialog box

Score Matrix Tab

The Score Matrix tab (Fig. 33.9) displays the matrix used to generate the current multiple alignment in AlignX Blocks. From this tab, you can also open other matrices for review or for application to the Blocks. Editing a matrix takes place in the Matrix Editor, accessible from AlignX and AlignX Blocks.

When a project is created, a residue substitution matrix from the corresponding protein template is used. If a template file is not found, the default matrix is used (blosum62mt2 for protein) or enter the name of a new score matrix in the Name text box. To review other matrices, click the **Select Matrix** button. From the Matrices file, select any other matrix to be loaded on the Score Matrix tab.

For further details regarding customizing or editing matrices, refer to Chapter 36.

Templates

Templates are the files that contain all the properties for the AlignX Blocks program. An AlignX Blocks project is created using default template file. You can also save current settings of the AlignX Blocks project in a template file or apply settings from existing template to the open project.

To save settings in a template choose **Edit > Setup > Save to File**. Enter or select the file to which settings will be saved.

To apply settings from existing template to the project that is already open choose **Edit > Setup > Load from File**. All the properties are applied after researching blocks.

Other AlignX Blocks Features

The following features can be performed in AlignX Blocks. Details are given in Chapter 3.

- Copying AlignX Blocks data to the clipboard
- Printing from AlignX Blocks
- Exporting molecules from AlignX Blocks
- Integrating AlignX Blocks with other Vector NTI applications and third-party tools on the WWW.

Appendix B References

AlignX-Basic Alignment Algorithms

Multiple Alignment

The alignment is created using the Clustal W algorithm (Nucleic Acid Research, 22 (22): 4673-4680, 1994). Briefly, a crude similarity between all pairs of sequences is calculated, called a "Pairwise alignment". These scores are then used to calculate a "guide tree" or dendrogram, which tells the multiple alignment stage the order in which to align the sequences for the final multiple alignment. Having calculated the dendrogram, the sequences are aligned in larger and larger groups until the entire sequences are incorporated in the final alignment.

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Appendix C ASCII Format; IUB Codes

Format for ASCII Sequence Files

An ASCII sequence file must obey the following rules:

- It must be a plain (ASCII) text file (not a file in WordPerfect, or MS Word formats).
- The file must contain the nucleotide (amino acid) sequence arranged in lines. Each line may contain the following:
- Nucleotide (amino acid) symbols and white space, or
- A number followed by white space and nucleotide (amino acid) symbols (therefore,, similar to GenBank format), in which case the number will be ignored, or
- A number only, in which case the number will be interpreted as a block of unknown nucleotides (amino acids) of the corresponding length.

IUB Formats recognized by Vector NTI

The following characters, defined by the International Union of Biochemistry (IUB), are used to represent nucleotides throughout Vector NTI:

<i>Symbol</i>	<i>Meaning</i>
A	adenine
T	thymine
C	cytosine
G	guanine
R	purine (A or G)
Y	pyrimidine (C or T)
W	A or T
S	C or G
M	A or C
K	T or G
B	C, G, or T
D	T, G, or A
H	C, A, or T
V	C, G, or A
N	unknown nucleotide

Table C. 1 IUB Formats for nucleotides

Appendix B References

AlignX-Basic Alignment Algorithms

Multiple Alignment

The alignment is created using the Clustal W algorithm (Nucleic Acid Research, 22 (22): 4673-4680, 1994). Briefly, a crude similarity between all pairs of sequences is calculated, called a "Pairwise alignment". These scores are then used to calculate a "guide tree" or dendrogram, which tells the multiple alignment stage the order in which to align the sequences for the final multiple alignment. Having calculated the dendrogram, the sequences are aligned in larger and larger groups until the entire sequences are incorporated in the final alignment.

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